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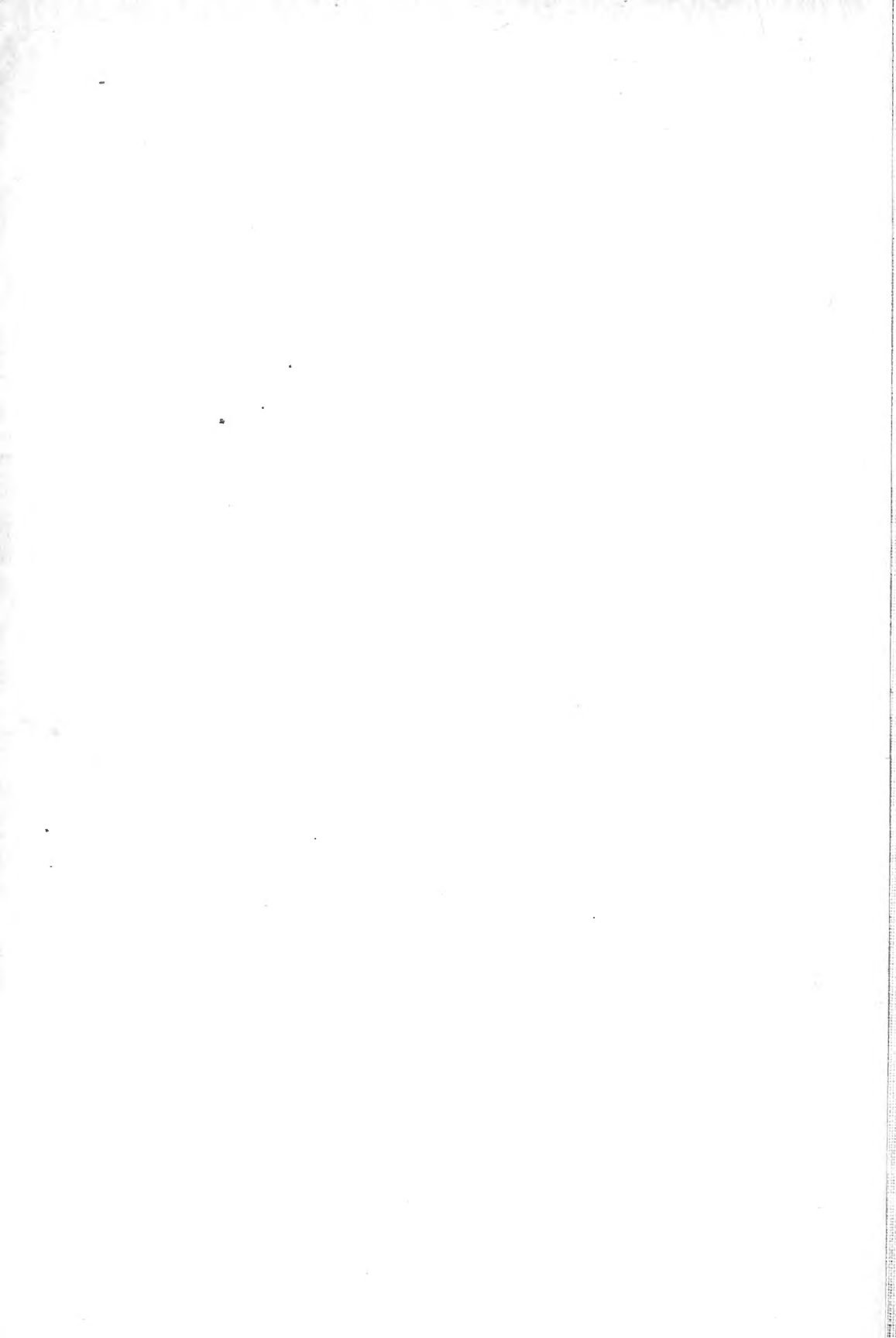
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DETERMINATION OF SEX IN HYDATINA SENTA

BY

DAVID DAY WHITNEY

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I INTRODUCTION

On account of the supposed influence of external factors in determining sex in *Hydatina senta*, this rotifer has attracted much interest in recent years. As is well known *Hydatina* produces three kinds of eggs, viz: (1) parthenogenetic eggs which develop into females; (2) smaller parthenogenetic eggs which develop into males; and (3) fertilized eggs which develop into females. Each female produces only one of these three kinds of eggs. Thus three types of females may be distinguished, viz: (1) females which produce females parthenogenetically, or female-laying females, ♀ ♀; (2) females which produce males parthenogenetically, or male-laying females, ♂ ♀; and (3) the sexual females that lay fertilized eggs.

Both female-laying females and male-laying females can be impregnated by males, but on the former, impregnation is supposed to have no effect. If the male-laying females are impregnated by the male in the first few hours after they leave the egg, such females produce fertilized eggs instead of parthenogenetic male eggs, thus showing that male-laying females can develop into sexual females that lay fertilized eggs.

The female-laying female can produce a family of daughter-females, some of which may lay female eggs and others may lay male eggs.

With the view of finding out the ratio in which these two classes of daughter-females are produced under various conditions I have carried out the experiments to be described.

Maupas found that a temperature of 26° to 28° C. would produce as high as 95 per cent of male-laying females while a temperature of about 14° C. would produce as low as 5 per cent of male-laying females.

Nussbaum, on the contrary, came to the conclusion that nutrition and not temperature is the sex controlling factor. He found that by starving the young females for the first few hours after they emerge from the egg they would produce a high percentage of males, but if they were fed at the time they leave the egg they produce a high percentage of females.

Punnett has carried out a few experiments along the lines laid down by Maupas and Nussbaum and finds that neither temperature nor nutrition is influential in determining the sex. He finds, on the contrary, that there are definite "sex strains." Some strains produce 40 to 50 per cent of males, others produce a very low percentage, 2 to 5 per cent, while others produce no males at all, although reared through as many as seventy-two generations.

The greater part of the work of the present paper was planned and begun in the spring of 1906, under the direction of Prof. T. H. Morgan, before the results of Punnett were published. Not knowing how to obtain proper food cultures the rotifers all died in July and the continuation of the experiments was deferred until October, 1906.

II MATERIAL AND METHODS

In the latter part of April, 1906, *Hydatina senta* was discovered in great numbers in a small pool on the Palisades of New Jersey near Grantwood. The pool was fed by a little stream or ditch which carried away the drainage from several cottages. The ditch was an extremely favorable place for the growth of *Euglena viridis* which collected in large patches on the sides and bottom. Immense numbers of *Euglena* floated down into the pool at the end of the ditch and served as food for the rotifers which abounded there in countless thousands. Sometimes as many as 150 to 250 individuals could be drawn up by a pipette in a few cc. of water.

About May 15 the pool dried up completely. The ditch still contained water but no rotifers were found in it after May 20. At this time there were innumerable larvæ of insects in the ditch and perhaps they exterminated the rotifers by feeding upon them.

In all experiments each individual female was isolated in a square or round watch glass which contained about 5 cc. of water and fed with *Euglena*, other protozoa and bacteria.

In order to obtain the *Euglena* and other protozoa a culture of horse manure and water (one to two ounces to a quart) was made, inoculated with *Euglena* and allowed to stand for two to three weeks at room temperature. At the end of this time the green coating of algæ, *Euglena*, etc., could be removed from the sides of the glass jar and served as an excellent food for the rotifers.

Great care was taken to keep these food cultures uncontaminated by rotifers. All watch glasses were placed in hot water after each experiment in order to destroy all eggs which adhered to the sides, thus preventing contamination of the following experiments by eggs of the preceding ones.

The experiments at temperature of 24° to 29° C. were conducted in an incubator. Those at a temperature of 20° to 22° C. were conducted on the laboratory tables at room temperature, while those at a temperature of 14° to 15° C. were carried on in an ice chest.

These rotifers are exceedingly hardy and can be very easily kept in the laboratory throughout the year. In May of 1906 a *Euglena* culture was prepared in a glass jar containing 2000 cc. of water

and a few rotifers put into it. The jar was covered so as to prevent evaporation of the water. Rotifers have lived in it to this time, April, 1907, although no more food material has ever been added. It is absolutely necessary that the surface of the water be free from a scum for the rotifers will die within a few hours if it is present. It is safer, in order to keep the surface free, to tie the horse manure in a muslin cloth and place it in a well covered jar nearly filled with water.

III INFLUENCE OF TEMPERATURE

I *Maupas' Experiments*

The experiments of Maupas were so briefly described that it is very difficult to understand clearly how he obtained his results.

Nussbaum and Punnett are inclined to believe he determined that a female was a male-laying or a female-laying individual by the size of the eggs that she produced. Small eggs being assumed always to give rise to males while larger eggs give rise to females. Nussbaum has measured a series of both male and female eggs and found that in some instances the two kinds of eggs over-lap in size. Thus he points out an error through which Maupas' results might have been obtained.

Isolating and counting the eggs of this rotifer would be exceedingly tedious and require almost constant attention. As the sexes can be readily distinguished at any period and as it requires only 36 to 48 hours for a female to mature and produce eggs it seems to me extremely probable that Maupas must have allowed some at least of the eggs to hatch before recording his results.

As his experiments are so few and briefly described it may be well to present them here in order that they may be compared with and interpreted by my own. Experiment I. Lot A, temperature 26° to 28° C. Five female-laying female sisters produced 104 eggs; 97 per cent developed into male-laying females. Lot B, temperature 14° C. Five other female-laying females, which were sisters of lot A, produced 260 eggs; 5 per cent developed into male-laying females.

Experiment II, temperature 14° C. Five female-laying females, kept from the time of hatching at this temperature, produced 110 eggs; 24 per cent developed into male-laying females.

The same five female-laying females were then placed at a temperature of 26° to 28° C. and produced 81 per cent male-laying females.

Experiment III, temperature 14° C. Six female-laying females which had been kept at this temperature from the time of hatching produced 34 eggs, of which 12 per cent developed into male-laying females.

The same six female-laying females were then placed at a temperature of 26° to 28° C. and allowed to produce 44 eggs, of which 95 per cent developed into male-laying females. These six females were alternately placed at 14° C. and 28° C. several times and always gave a high percentage of male-laying females at the higher temperature.

2 *Author's Experiments*

a Temperature 20° to 22° C.

Experiment I, October 24, 1906. A female-laying female was isolated from a jar which was stocked with rotifers collected October 2, from the same pool in which the animals were found in the preceding spring.

This strain was carried through twelve generations and the percentage of male-laying females determined. Each female was supplied with an abundance of food from the time of hatching, isolated in a separate watch-glass, and kept upon the laboratory table at room-temperature.

Table I gives the ratio of the mother individuals producing male and female offspring in the 3264 daughter-females of 95 female-laying females in the twelve generations.

This experiment was made in order to obtain the percentage of male-laying females produced at room temperature of 20° to 22° C., in order to be able to have some standard percentage of male-laying females with which to compare the results of the experiments conducted at lower and higher temperatures.

TABLE I

Record of the production of male-laying and female-laying females among the 3264 daughter-females of 95 female-laying mothers.

Temperature 20° to 22° C.

Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
I	1	31	15	16	48+	X	3	32	8	24	25
							4	15	3	12	20
II	1	28	0	28	0		5	43	8	35	18+
	2	53	8	45	15+		6	48	11	37	22+
	3	27	4	23	14+		7	25	0	25	0
III							8	38	15	23	39+
	1	44	17	27	38+		9	17	4	13	23+
	2	46	14	32	30+		10	48	15	33	31+
IV							11	48	16	32	33+
	1	51	1	50	1+		12	43	4	39	9+
	2	47	15	32	31+		13	44	10	34	22+
	3	52	16	36	30+		14	49	14	35	28+
	4	35	7	28	20		15	45	18	27	40
V							16	16	6	10	37+
	1	47	0	47	0		17	45	4	41	8+
	2	44	2	42	4+		18	50	7	43	14
	3	41	1	40	2+		19	32	4	28	12+
	4	45	0	45	0		20	21	0	21	0
VI							21	21	4	17	19+
	1	32	12	20	37+		22	20	2	18	10
	2	45	4	41	8+		23	25	0	25	0
	3	36	0	36	0	XI	1	49	8	41	16+
	4	47	7	40	14+						
VII	5	48	22	26	45+	XII	1	38	3	35	7+
							2	45	9	36	20
	1	30	15	15	50		3	48	4	44	8+
	2	35	1	34	2+		4	31	5	26	16+
	3	27	0	27	0		5	39	13	26	33+
VIII	4	24	0	24	0		6	31	10	21	32+
	5	19	5	14	26+		7	54	10	44	18+
							8	45	6	39	13+
	1	41	16	25	39+		9	35	11	24	31+
	2	17	1	16	5+		10	43	5	38	11+
IX	3	47	18	29	38+		11	38	3	35	7+
	4	26	0	26	0		12	27	9	18	33+
	5	38	0	38	0		13	42	16	26	38+
							14	34	1	33	2+
	1	24	1	23	4+		15	43	15	28	34+
X							16	37	6	31	16
	1	49	13	36	26+		17	31	5	26	16+
	2	45	10	35	22+						

TABLE I—Continued

Temperature 20° to 22° C.

Gen.	No. ♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	No. ♂ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
XII	18	26	7	19	22+	XII	30	16	5	11	31+
	19	33	3	30	9+		31	9	1	8	11+
	20	44	3	41	6+		32	18	8	10	44+
	21	20	0	20	0		33	20	6	14	30
	22	45	9	36	20		34	47	14	33	29+
	23	31	11	20	35		35	19	4	15	21+
	24	28	10	18	35+		36	26	8	18	30+
	25	15	3	12	20		37	11	0	11	0
	26	34	1	33	2+		38	37	11	26	29+
	27	15	0	15	0		39	34	19	15	55+
XII	28	14	3	11	21+		40	25	6	19	24
	29	6	1	5	16+		41	35	4	31	11+

The nature of the sex-producing power of the daughter-females of each individual mother is given separately in order to show that the ratio between the daughter-females producing male and female offspring varies with different mother-individuals, and also varies as much in the daughter females of sister-mother-individuals.

TABLE II

Summary of each generation in Table I and also the final summary of all the generations taken together.

Temperature 20° to 21° C.

Gen.	♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀	Gen.	♀ ♀ mother	Eggs laid	Offspring		Per cent ♂ ♀
			♂ ♀	♀ ♀					♂ ♀	♀ ♀	
I	1	31	15	16	48+	VII	5	135	21	114	15+
II	3	108	12	96	11+	VIII	5	169	35	134	20+
III	2	90	31	59	34+	IX	1	24	1	23	4+
IV	4	185	39	146	21+	X	23	819	176	643	21+
V	4	177	3	174	1+	XI	1	49	8	41	16+
VI	5	208	45	163	21+	XII	41	1269	268	1001	21+
							95	3264	654	2610	20+

individual, strain IV, and six generations from the other individual, strain III, were recorded. Six generations were kept at a temperature of 25° to 26° C. and two generations at 26° to 29° C.

Table III gives the results of the experiment obtained at this higher temperature. The 26 female-laying mothers from eight generations, and from two strains, produced 208 daughter-females of which 22 + per cent were male-laying females. This percentage is practically the same as that obtained at room temperature.

c Temperature 14° to 15° C.

Experiment III, October 8. A female-laying female was isolated from stock jar as in Experiment I, and placed in an ice chest. A record of only a few of her offspring was kept and is shown in Table IV.

TABLE IV

Record of the production of male-laying and female-laying females among the 167 daughter-females of 7 female-laying mothers.

Temperature 14° to 15° C.

No. of ♀ ♀ mother	Eggs laid	Offspring			Per cent ♂ ♀
		Fed	♂ ♀	♀ ♀	
1	48	47	11	36	23+
2	50	28	4	24	14+
3	30	20	3	17	15
4	22	9	2	7	22+
5	41	19	5	14	26+
6	36	15	0	15	0
7	51	29	10	19	34+
7	278	167	35	132	20+

Out of 167 daughter-females from 7 different mothers 20 + per cent were male-laying females. The mother-individuals were reared at this low temperature as well as the daughter-females.

The percentage of male-laying females is about the same as that obtained at temperatures 20° to 22° C. and 25° to 29° C.

The foregoing results agree with those obtained by Nussbaum and Punnett but seem contrary to Maupas' results.

IV THE RELATIVE NUMBER OF EGGS WHICH A MALE-LAYING FEMALE AND A FEMALE-LAYING FEMALE PRODUCE

It seems evident from Maupas' account of his own experiments that he did not isolate each female-laying mother and each one of her daughter-females but kept the female-laying mothers together in one dish and their daughter-females together in another dish.

If it is assumed that Maupas made no mistake in determining the sex character of the eggs before they hatched, or even that he allowed all eggs to hatch before he recorded their sex character, his results can be easily explained.

TABLE V

The number of eggs laid by each of 13 sisters, of which 6 were male-laying and 7 were female-laying, showing that the average number of eggs laid by each of the two kinds of females is very nearly the same.

Temperature 20° to 22° C.
13 sisters

♂ ♀ Mother	♀ Eggs	Av.	♀ ♀ Mother	♀ Eggs	Av.
1	46		1	47	
1	50		1	38	
1	47		1	37	
1	46		1	43	
1	41		1	38	
1	31		1	45	
			1	38	
6	261	43½	7	268	40½

He gives no results of experiments conducted at a temperature midway between 14° and 28° C. but only results obtained at these two extremes. The results that were obtained at 14° C. may very likely be identical with those that could have been obtained at a room temperature around 20° C.

Maupas recognized the fact that male-laying females produce eggs faster than female-laying females but makes no mention of the number of eggs that each kind of female may produce at different temperatures. He seems to assume that they always pro-

duce about an equal number, 40 to 50 each, but the following experiments will show the error of this assumption.

1 *Temperature 20° to 22° C.*

Experiment IV, November 5. Of 13 sister individuals kept at room temperature and with the same amount of food 6 produced male eggs and 7 produced female eggs. The average number of eggs produced by each female was nearly the same. The results are shown in Table V.

2 *Temperature 24° to 29° C.*

Experiment V, November 9. Three lots of sister-individuals from three different mother-individuals were kept in an incubator

TABLE VI

Record of the number of eggs laid by 11 sisters, of which 6 were male-laying and 5 were female-laying, showing that the average number of eggs produced by the male-laying females is about two times as great as the average number produced by the female-laying females.

Temperature 24° to 25° C.

11 sisters

♂ ♀ Mother	♂ Eggs	Av.	♀ ♀ Mother	♀ Eggs	Av.
1	37		1	16	
1	35		1	17	
1	26		1	16	
1	38		1	11	
1	26		1	10	
1	22				
6	184	30 $\frac{2}{3}$	5	70	14

and the number and sex character of the eggs that each produced was very carefully noted. The results are shown in Tables VI, VII and VIII. The records of the individuals in Tables VI and VII were taken at the same temperature of 24° to 25° C. while those of Table VIII were taken at a higher temperature of 26° to 29° C.

These tables show a decided change in the ratio between the number of eggs produced by a male-laying female and a female-laying female. As the temperature is raised the female-laying

TABLE VII

Record of the number of eggs laid by 21 sisters, of which 9 were male-laying and 12 were female-laying, showing the average number of eggs produced by the male-laying females is about two times as great as the average number produced by the female-laying females.

Temperature 24° to 25° C.

21 sisters

♂ ♀ Mother	♂ Eggs	Av.	♀ ♀ Mother	♀ Eggs	Av.
1	25		1	6	
1	39		1	14	
1	34		1	16	
1	37		1	11	
1	28		1	10	
1	25		1	12	
1	29		1	12	
1	23		1	9	
1	22		1	17	
			1	14	
			1	16	
			1	21	
9	261	29 $\frac{1}{3}$	12	158	13 $\frac{1}{6}$

TABLE VIII

Record of the number of eggs laid by 14 sisters, of which 2 were male-laying and 12 were female-laying, showing that the average number of eggs produced by the male-laying females is nearly four times as great as the average number produced by the female-laying females.

Temperature 26° to 29° C.

14 sisters

♂ ♀ Mother	♂ Eggs	Av.	♀ ♀ Mother	♀ Eggs	Av.
1	23		1	3	
1	19		1	5	
			1	1	
			1	8	
			1	6	
			1	8	
			1	3	
			1	5	
			1	4	
			1	4	
			1	9	
			1	10	
2	42	21	12	66	5 $\frac{1}{2}$

females produce fewer and fewer eggs while the decrease in the number of eggs produced by male-laying females is not as great.

Table IX shows a rough approximation of the ratio in which the male and female eggs are produced at these different temperatures.

TABLE IX

The approximate ratios in which the males and females are produced at different temperatures, as seen in Tables V-VIII.

♂	♀	
1	1	Temp. 20° to 22° C. Table V
2	1	Temp. 24° to 25° C. Table VI-VII
4	1	Temp. 26° to 29° C. Table VIII

From the foregoing experiments and tables it is evident that temperature has nothing to do directly with determining sex in *Hydatina senta* but indirectly it determines the number of each sex produced by regulating the number of eggs that each kind of female lays. At a temperature of 20° to 22° C. the male-laying and female-laying females lay about the same number of eggs each, but at a higher temperature of 26° to 29° C. the male-laying females lay about four times as many as the female-laying females.

V EARLY PRODUCTION OF MALE-LAYING FEMALES IN A FAMILY OF DAUGHTER-FEMALES

None of the previous workers with *Hydatina senta* have isolated the eggs of a female-laying female in the order in which they were produced to determine whether there is any tendency for the earlier laid eggs to produce more male-laying females than the later laid eggs.

In my experiments in which all the daughter-females of each individual mother were carefully isolated and the sex character of their immediate offspring was recorded it is clearly shown that the male-laying daughter females appear among the earlier ones in the family rather than among the later ones.

In Diagram 1 the plotted line indicates the production of male-laying females among the 472 daughter-females of eleven mother-

individuals, each one of which produced 40 to 44 (average $42\frac{10}{11}$) eggs. The eggs were allowed to hatch in the dish with each mother and the young daughter-females isolated soon after hatching. Their different sizes would indicate their relative ages and thus the approximate order in which the eggs were produced. The young daughter-females were isolated in lots from 1 to 8. This manner of isolation is subject to some error but on the whole gives a fairly good approximation of the truth.

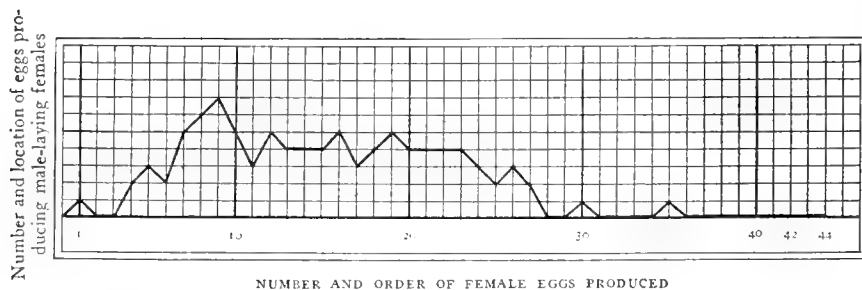


Diagram 1 Record of the egg production of 11 female-laying females from Table I, showing in which part of the egg laying period the male-laying females were produced. Each female laid 40 to 44 (average $42\frac{10}{11}$) eggs. Of the 472 daughter-females 20+ per cent were male-laying females.

Nearly all of the male-laying females were produced among the first 28 eggs laid. Only two male-laying females were produced from the twenty-ninth to the forty-second laid eggs. Of the daughter-females 20+ per cent were male-laying females.

Diagram 2. This is to show the same point as Diagram 1.

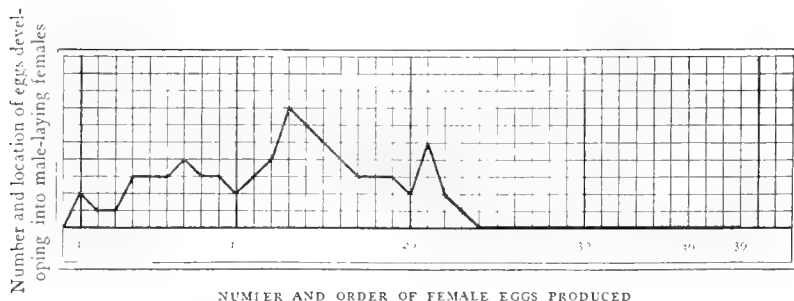


Diagram 2 Record of the egg production of 12 female-laying females from Table I, showing in which part of the egg laying period the male-laying females were produced. Each female laid 35 to 39 (average $36\frac{1}{2}$) eggs. Of the 441 daughter-females 16+ per cent were male-laying.

Twelve other female-laying females, each of which laid 35 to 39 (average 36+) eggs, produced all their male-laying daughter-females among the first twenty-four eggs laid. This is a clearer case than Diagram 1, because there are no scattering male-laying females among the later produced eggs. Of the daughter-females 16+ per cent were male-laying females.

In these two diagrams the mother-individuals were not specially selected but the record of all mothers, in Table I, producing 40 to 44 daughter-females, is shown in one diagram and the record of all mothers, in Table I, producing 35 to 39 daughter-females is shown in the other diagram. The numbers 35 to 39 and 40 to 44 were chosen because they seemed more likely to be the normal than a higher one.

These results, together with those obtained at different temperatures throw a great deal of light upon Maupas' results. In his experiments the highest percentages of males was always obtained from mothers which developed from early laid eggs.

In Table I it is seen that an individual mother may produce 0 to 40+ per cent of daughter male-laying females. This fact must also be taken into account when explaining Maupas' few experiments.

VI INFLUENCE OF FOOD

Nussbaum supported Maupas' conclusions that external factors can change the sex ratio in *Hydatina* but explains this change as being due to poor nutrition of the females and not due directly to the influence of temperature. At the higher temperature the processes of metabolism are taking place so rapidly that the animals cannot eat food and assimilate it fast enough to prevent their tissues from being in a semi-starved condition. Nussbaum's experiments seemed to show evidence that young females which are starved for several hours as soon as they leave the egg produce a higher percentage of males than those that are fed from the moment they hatch.

In many of his experiments he kept many individual females together and did not follow the history of each individual separately.

Punnett has pointed out that all the starved females of Nussbaum's experiments did not produce males, which invalidates his general conclusions.

Punnett has isolated female eggs of a "pure female strain," and after they hatched starved the young females from 2 to 20 hours but no males ever appeared, although the young females were starved for several consecutive generations.

I have followed the history of many females which have been starved for several hours immediately after hatching at a temperature ranging from 14° to 29° C. and have found no trace of evidence that a higher percentage of male-laying females is produced.

I Temperature 20° to 22° C.

Experiment I. Sixty-two eggs were selected at random from the sets of eggs produced by four female-laying females. They

TABLE X

Record of the sex character of the eggs laid by 27 sisters, 15 of which were without food for the first 6 to 26 hours after hatching and 12 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	6		♀
2	10		♀
5	11		♀
1	12		♀
1	16		♀
2	21		♀
1	23	♂	
1	26	♂	
2	fed	♂	
10	fed		♀

TABLE XI

Record of the sex character of the eggs laid by 45 sisters, 11 of which were without food for the first 21 to 26 hours after hatching and 34 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
11	21-26		♀
13	fed	♂	
21	fed		♀

were placed in "Great Bear" spring water, such as is sold in New York City for drinking purposes, and allowed to hatch. After

hatching each daughter-female was kept in this water without food from 6 to 71 hours.

Of 109 daughter-females from the same mother as the above, 62 were well supplied with food from the moment they hatched.

Tables X to XIII give the detailed results and Table XIV gives the summary. The mother-individuals of Tables X and XII

TABLE XII

Record of the sex character of the eggs laid by 53 sisters, 31 of which were without food for the first 11 to 59 hours after hatching and 22 were abundantly supplied with food from the moment they hatched.

Temperature 20 to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
1	11		♀
1	13		♀
2	14		♀
1	15		♀
1	21		♀
5	36	♂	
7	36		♀
1	38	♂	
2	38		♀
1	42		♀
1	47	♂	
4	47		♀
2	50		♀
1	54		♀
1	59		♀
1	fed	♂	
21	fed		♀

TABLE XIII

Record of the sex character of the eggs laid by 46 sisters, 5 of which were without food for the first 50 to 71 hours after hatching and 41 were abundantly supplied with food from the moment they hatched.

Temperature 20° to 22° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	50	♂	
1	50		♀
1	71		♀
1	71	♂	
11	fed	♂	
30	fed		♀

were sisters. The 11 sisters-individuals of Table XI were starved in filtered boiled spring water placed in sterilized test tubes with cotton stoppers.

The percentage of male-laying females among all the starved daughter-females is slightly lower than that of those which were fed.

TABLE XIV

Summary of Tables X to XIII, showing the percentage of male-laying females that occurred among the females which were without food for the first 6 to 71 hours after hatching, and also the percentage of male-laying females which occurred among the females that were abundantly supplied with food from the moment they hatched.

<i>Temperature 20° to 22° C.</i>				
Indi- viduals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
	<i>hours</i>			
62	6-71	12	50	19 +
109	fed	27	82	24 +
171		39	132	22 +

2 *Temperature 14° to 15° C.*

Experiment II, October 29. Two female-laying females were reared at this temperature and their daughter-females isolated. Thirty-five daughter-females were without food from 11 to 64 hours after they left the egg, and 49 were abundantly supplied with food as soon as they hatched.

The detailed results are shown in Tables XV and XVI while Table XVII gives the summary.

The difference between the percentage of males produced by those starved and those fed is not very great and probably means nothing.

3 *Temperature 25° to 26° C.*

Experiments III, October 31. Twenty-six female eggs from several individuals were produced at this temperature and as soon as they hatched the young females were starved from 1 to 13 hours. 19+ per cent of these starved females produced male eggs.

Tables XVIII and XIX give the detailed history and Table XX gives the summary.

These three experiments, including Tables X to XX, clearly demonstrate that food has no influence in determining whether a female shall produce male or female offspring.

TABLE XV

Record of the sex character of the eggs laid by 49 sisters, 19 of which were without food for the first 11 to 41 hours after hatching and 30 were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Sister-individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	hours		
4	11		♀
1	20		♀
2	20	♂	
3	21		♀
1	23		♀
5	25		♀
1	25	♂	
1	27		♀
1	41		♀
4	fed	♂	
26	fed		♀

TABLE XVI

Record of the sex character of the eggs laid by 35 sisters, 16 of which were without food for the first 20 to 64 hours after hatching and 19 were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Sister-individuals	Starved from time of hatching	Character of egg produced	
		♂	♀
	hours		
2	20		♀
1	21	♂	
2	21		♀
2	23		♀
1	46	♂	
1	46		♀
1	48		♀
1	53	♂	
1	53		♀
1	58		♀
1	61	♂	
1	61		♀
1	64	♂	
5	fed	♂	
14	fed		♀

VII MALE AND FEMALE STRAINS

Punnett says: "My experiments have led me to the conclusion that among the rotifers I used were certainly three different types of thelytokous (female-laying) females, viz:

A Females producing a high percentage of arrenotokous (male-laying) females.

B Females producing a low percentage of arrenotokous females.

C Purely thelytokous females producing no arrenotokous females" (p. 226).

TABLE XVII

Summary of Tables XV to XVI, showing the percentage of male-laying females that occurred among the females which were without food for the first 11 to 64 hours after hatching, and also the percentage of male-laying females which occurred among the females that were abundantly supplied with food from the moment they hatched.

Temperature 14° to 15° C.

Individuals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
35	11-64	8	27	22 +
49	fed	9	40	18 +
84		17	67	20 +

TABLE XIX

Record of the sex character of the eggs laid by 19 individuals females which were without food for the first 1 to 13 hours after hatching.

Temperature 25° to 26° C.

Individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
1	1		♀
2	2		♀
1	3	♂	
3	3		♀
1	4	♂	
1	5	♂	
2	5		♀
2	6		♀
1	8	♂	
3	10		♀
2	13		♀

TABLE XVIII

Record of the sex character of the eggs laid by 15 sisters, 7 of which were without food for the first 7 to 13 hours after hatching and 8 were abundantly supplied with food from the moment they hatched.

Temperature 25° to 26° C.

Sister individuals	Starved from time of hatching	Character of eggs produced	
		♂	♀
	<i>hours</i>		
2	7		♀
2	10		♀
1	10	♂	
2	13		♀
1	fed	♂	
7	fed		♀

TABLE XX

Summary of Tables XVIII to XIX, showing the percentage of male-laying females that occurred among the 26 females that were without food for the first 1 to 13 hours after hatching.

Temperature 25° to 26° C.

Individuals	Starved from time of hatching	Character of eggs produced		Per cent ♂ ♀
		♂	♀	
	<i>hours</i>			
26	1-13	5	21	19 +

Punnett realized that these conclusions were based on rather scanty data. His data can be shown to be entirely insufficient. His type *A* is based upon only one experiment which extended through 23 generations and included 109 individuals. 42 + per cent of these 109 individuals were male-laying females.

Type *C* is based upon much more evidence, but it is not sufficient to warrant a decisive conclusion.

In October, 1906, I started a strain or pedigree culture which extended through 62 generations including 167 mother female-laying females and 3959 daughter-females. This strain was kept at room temperature of 20° to 22° C. Its history is recorded in Tables I and XXI.

Table I. Out of 3264 daughter-females from 95 mother-individuals which extended through 12 generations 20 + per cent were male-laying females.

Table XXI. In 15 generations, XIII to XXVIII, including 76 daughter-females from 15 mother-individuals only 9 + per cent were male-laying females.

In 17 generations, XXIX to XLV, including 208 daughter-females from 17 mother-individuals no male-laying females appeared. In generation XLVI the first 327 daughter-females from 18 mother-individuals yielded 48 + per cent of male-laying females.

The next 11 generations XLVII to LVII including 58 daughter-females from 11 mother-individuals gave 29 + per cent male-laying females.

These results show that a strain producing a higher percentage of male-laying females can develop into a strain yielding a much lower percentage, or even into a strain yielding no male-laying females at all. Furthermore, the apparently pure female-laying female strain can develop into one which will give a very high percentage of male-laying females.

Thus the three strains or types of Punnett can be found in one strain and each is capable of giving rise to the other types according as the data is scanty or extensive.

The high percentage of male-laying females in generation XLVI can be readily explained by the results shown in Diagrams 1 and 2 which clearly demonstrate that the male-laying females are pro-

duced earlier in a set of eggs than are the majority of the female-laying females. In this generation the 18 mothers produced only an average of $18 +$ eggs each, because the experiment was discontinued at this point.

TABLE XXI

Continuation of the strain of which the beginning is recorded in the 12 generations of Table I.

Gen.	♀ ♀ Mother	Daugh- ter ♀ isolated	♂ ♀	♀ ♀	Gen.	♀ ♀ Mother	Daugh- ter ♀ isolated	♂ ♀	♀ ♀
XIII	1	6	1	5	XXXVIII	1	37	0	37
XIV	1	6	1	5	XXXIX	1	6	0	6
XV	1	6	0	6	XL	1	26	0	26
XVI	1	6	0	6	XLI	1	25	0	25
XVII	1	2	0	2	XLII	1	14	0	14
XVIII	1	1	0	1	XLIII	1	18	0	18
XIX	1	5	0	5	XLIV	1	17	0	17
XX	1	6	0	6	XLV	1	18	0	18
XXI	1	6	0	6	XLVI	18	327	160	167
XXII	1	6	3	3	XLVII	1	1	0	1
XXIII	1	5	0	5	XLVIII	1	6	4	2
XXIV	1	6	0	6	XLIX	1	6	3	3
XXV	1	6	0	6	L	1	6	2	4
XXVI	1	6	1	5	LI	1	6	0	6
XXVII	1	1	0	1	LII	1	6	0	6
XXVIII	1	2	1	1	LIII	1	6	0	6
XXIX	1	5	0	5	LIV	1	6	5	1
XXX	1	6	0	6	LV	1	5	2	3
XXXI	1	4	0	4	LVI	1	6	1	5
XXXII	1	1	0	1	LVII	1	4	0	4
XXXIII	1	5	0	5	LVIII	3	6	0	6
XXXIV	1	6	0	6	LIX	1	6	0	6
XXXV	1	6	0	6	LV	2	6	0	6
XXXVI	1	6	0	6	LXI	2	6	0	6
XXXVII	1	6	0	6	LXII	2	4	1	3

How a seemingly pure female-laying female strain is obtained when only a few individuals are isolated from each generation while a parallel strain does not yield the same results is not yet clear. It may be due to some trick of selection in isolating the young females of each generation.

If a large number, 45 + per cent, of the daughter-females of one mother produce males it does not necessarily follow that a high or a low percentage of the daughter-females of the next generation will produce males. Nor does it seem to be true that if the daughter-females of one generation produce all female offspring that the daughter-females of the following generation will do so. Table XXII shows the history of both classes of daughter-females in five generations.

TABLE XXII

Record of all the female-laying females that were isolated in five consecutive generations, showing that there is no constant relationship between the percentage of male-laying females that are produced by a mother and the percentage of male-laying females that are produced by the daughter-female in the next generation.

Gen.	Sisters	Off- spring ♂ ♀	Moth- er ♀ ♀	Off- spring ♀ ♀	Gen.	Sisters	Off- spring ♂ ♀	Moth- er ♀ ♀	Off- spring ♀ ♀
I			1.		V	5	15		15
II		16	 1.	36			1		34
							0		27
III	4	0		45			0		24
		2		42			5		14
		1		42					
		0	 1.	47					
IV	5	12		20					
		4		41					
		0		36					
		7		40					
		22	 1.	26					

VIII THE PRODUCTION OF FERTILIZED EGGS

The winter or fertilized egg is supposed to be the male parthenogenetic egg which has been fertilized. This produces a female. The egg has much more yolk material and a thicker shell than the male parthenogenetic egg. A female produces from twelve to twenty fertilized eggs, while a male-laying female produces from forty to fifty parthenogenetic eggs. In order to obtain fertilized eggs males must copulate with very young male-laying females.

In a few experiments, comprising several hundred females which had copulated with males when very young, Maupas found that the percentage of females producing fertilized eggs was the same as the percentage of male-laying females from several hundred females that never had copulated with males. He concluded that the fertilized egg is the male parthenogenetic egg which has been fertilized.

I have repeated his experiments on a smaller scale and the same results were obtained. Furthermore, the producers of fertilized eggs appeared among the early laid eggs of the mother-individuals.

Diagram 3 shows the occurrence of the layers of fertilized eggs among their sister-individuals from five mothers which produced 125 eggs.

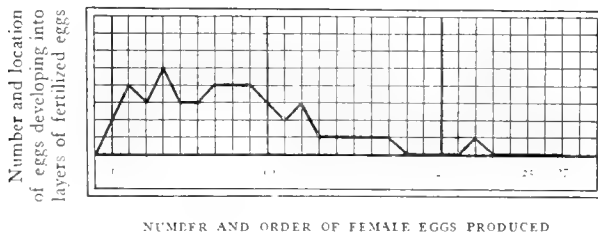


Diagram 3 Record of the egg production of five female-laying females showing in which part of the egg-laying period the layers of fertilized or "winter eggs" were produced. Each female laid 21 to 27 (average 25) eggs. Of the 125 daughter-females 36 + per cent laid fertilized eggs.

Many males, fifteen to twenty, were constantly kept in the dishes with each of the five mothers, so that as each daughter-female emerged from the egg there were many males present.

Only one male-laying female appeared among the 125 daughter-females. This experiment was conducted in the fourteenth generation of the strain in Tables I and XXI, soon after the isolation of the large numbers in generation XII which gave 21 + per cent of male-laying females.

Of the 125 daughter-females 36 + per cent produced fertilized eggs. This percentage is high because the mother-females produced, on an average, only twenty-five eggs each, but if they had produced forty eggs each the percentage would have fallen to

about twenty-three, provided that there had been no more mothers of fertilized eggs to have been produced. The Diagram 3 shows only one occurring between the eighteenth and twenty-seventh egg.

The number and order of occurrence of the mothers of fertilized eggs together with the number and occurrence of the layers of male eggs in parallel sets of daughter-females seem to indicate that the layer of male eggs and the layers of fertilized or winter eggs are identical at one stage of their life.

In another species of rotifer, *Asplancha*, Lauterborn has observed winter eggs and male embryos in the same individual. Among the *Daphnia*, Issakowitsch has found that the same female may produce winter eggs and male eggs.

Therefore it is not unreasonable to suppose that the immature male-laying female of *Hydatina senta* is capable of developing into a layer of fertilized eggs or a layer of male eggs, according to the impregnation or lack of impregnation by the male.

IX SUMMARY

1 Temperature has no influence in determining the sex of *Hydatina senta*.

2 About 22 per cent of the females at any temperature from 14° to 29° C. are male-laying.

3 A male-laying female produces eggs faster than a female-laying female and at a temperature of 25° to 29° C. a male-laying female produces more eggs throughout her lifetime than a female-laying female.

4 The male-laying females occur in the early part of a family of daughter-females.

5 Starving the young females for the first few hours after they hatch does not cause them to produce a higher percentage of male eggs.

6 There are no strains that constantly produce a high or a low percentage of male-laying females.

7 The "pure female-laying female strain" can give rise to the normal percentage, $22 \pm$, of male-laying females.

8 The male-laying female may produce fertilized or winter eggs, provided that she has been impregnated by a male at the proper time.

Zoological Laboratory

Columbia University

May 1, 1907

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A NEW EXPLANATION OF THE MECHANICS OF MITOSIS

BY

ARTHUR B. LAMB

WITH TWO FIGURES

The almost universal recurrence of essentially the same regular arrangement of the chromatin substance in dividing cells indicates emphatically that the same very definitely acting force or complex of forces is operative in them all. Numerous suggestions have been made as to what this omnipresent force may be, but none of them have been able to meet the many requirements of the problem. Moreover, our real knowledge of the whole matter is so scanty that any explanation seems at present a little premature. I am, nevertheless, going to offer still another explanation of this phenomenon; first, because it may prove suggestive to others and may prompt fresh observation, and second, because it calls attention to a phenomenon which deserves the consideration of cytologists, whether it has any application to the present case or not.

The marked polarity which mitotic figures exhibit, best described by saying that they resemble the configuration assumed by iron filings between unlike magnetic poles, together with the movements which the chromatin substances execute about one another oblige us to believe that this unknown force is of a polar nature, that is, acts outward from a center and exerts its influence at a distance. The only objection to this conclusion is that a crossing of astral rays has been observed. The lines of force in the field of any polar force cannot, however, cross, and consequently the astral rays which would be assumed to follow these lines of force also cannot, or should not, cross. This crossing, though certainly real, is not, apparently, the prevailing condition, and it can be

explained on the assumption of an intermittent or non-synchronous activity of the centers, as Reinke¹ has shown.²

Assuming, then, the existence of some polar force exerting its action at a distance, we are confronted with two possible alternatives regarding the sign of this action. That is, we may imagine either that the centrosomes attract, or that they repel each other. Wilson³ has urged that the astral centers represent centers of *traction*, caused, perhaps, by a volume change at those places. This is in entire agreement with the configuration assumed by the astral rays and the spindle fibers. They simulate the magnetic field between opposite poles, as pointed out above. But this view is quite at variance with the actual movements of the centrosomes. They move apart, even at a stage when astral rays are well developed and hence seem to repel each other and not attract as they ought if they represent *opposite* poles. Lillie⁴ adopts the other alternative, as did Meves.⁵ He considers the astral centers to repel each other. In this way he explains the movements of the centrosomes satisfactorily enough, but is confronted by the difficulty of accounting for the configuration of the astral rays. Lillie assumes that electric charges located on the centrosomes are the particular forces which produce the repulsion. He would explain the unexpected configuration of the fibers and the astral rays by the rather dubious assumption of a localized positive inter-astral area which superposes its effect on the purely repellent action of the astral centers. Looking at the matter more closely we see that for every unit of negative electricity on the chromatin substance there should be a corresponding unit of positive electric-

¹ Reinke, Fr.: Arch. f. Entwicklungsmech., ix, 1900.

² Rhumbler: *Ibid.*, iii, iv and v, 1896, 1897 and 1899, has suggested a non-polar force to explain the astral rays independently progressing rays of crystallization out of a supersaturated solution. While avoiding the difficulty of crossed fibers this explanation encounters the still more formidable one of accounting for the universal occurrence of *curved* fibers.

³ *Ibid.*, xiii, p. 354-395, 1901. See also his book, *The Cell in Development and Inheritance*, 3d Ed. The Macmillan Company, New York. It is a pleasure to express my thanks for a most profitable discussion of this whole question with Professor Wilson, who, it seems, had already considered the possibility of a hydrodynamic explanation.

⁴ Amer. Jour. Physiol., xv, 46-84, 1905.

⁵ Ergebn. d. Anat. u. Entwickl., vii, viii. Merkel u. Bonnet.

ity in or on the surrounding aqueous solution. Moreover, since this aqueous solution contains inorganic, ionized salts it must be a conductor of electricity, and the positive charge must be distributed over the whole solution. Any localized positively charged area in the electrolyte, except for the supposed "double layer" around each charged particle seems, consequently, unlikely. Lillie has encountered a similar difficulty in accounting for the configuration of the chromosomes. They ought not only to be repelled from the astral centers but also to migrate toward the boundary of the equatorial plate. This latter thing they do not do, and Lillie is therefore again obliged to make the assumption of a localized positive inter-astral region.

There is, however, another force which might well come into play here, which so far as I know has not been mentioned in this connection before, and which involves none of the objections urged against the electrostatic explanation. I refer to the mutual repulsions and attractions, exerted by bodies pulsating or oscillating in a fluid medium. We owe our knowledge of this branch of hydrodynamics chiefly to the two Bjerknes, father and son.⁶ They have shown that bodies pulsating or oscillating synchronously in a liquid attract or repel one another depending on whether they pulsate or oscillate in the same or opposite phase. Furthermore, these bodies set up lines of flow in the liquid, real hydrodynamic lines of force, which simulate exactly the lines of force in magnetic or electric fields. The sign of this force is, however, in general, just the reverse of that in electric or magnetic fields. Bodies pulsating synchronously and in opposite phase repel each other, *although the form of the field they produce is identical with that between unlike magnetic poles which attract each other*. Similarly, two spheres oscillating synchronously and in the same phase repel each other, although they too produce a field like that between opposite magnetic poles. The following experimentally derived figures, taken from Bjerknes' text-book, illustrate this identity of form and reversal of sign in the electric and hydrodynamic energies:

⁶ See *Hydrodynamische Fernkräfte*, v. Bjerknes; 2 vols. Leipzig, J. A. Barth, 1902.

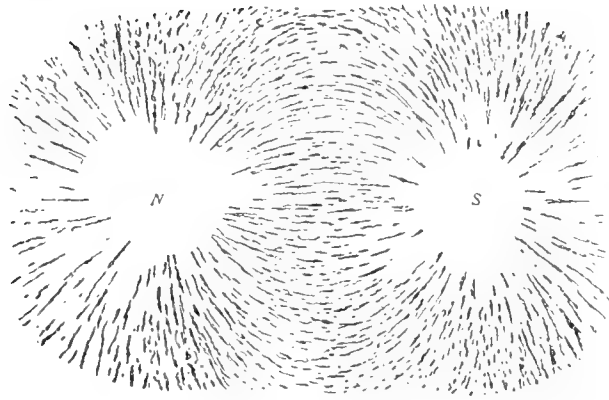


Fig. 1 Lines of force between unlike magnetic poles. REPULSION.

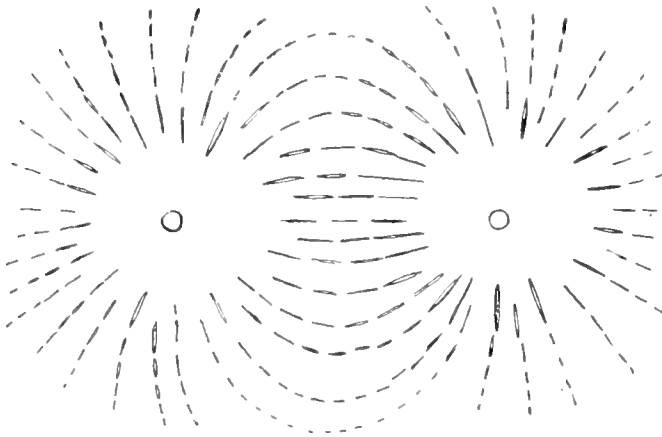


Fig. 2 Lines of flow between oppositely pulsating bodies. ATTRACTION.

It is this exact reversal of sign of hydrodynamic action at a distance, as compared with electric and magnetic actions, which makes this force peculiarly applicable to the case of mitotic figures, obviating many objections which beset the previous explanations, and particularly the fact that with previously considered polar forces if one made an assumption which would explain the *form of the field*, the *motion* of the centrosomes appeared contradictory

and vice versa. If, however, we assume that the centrosomes are pulsating synchronously and in opposite phases, or oscillating synchronously and in the same phase, we obtain the desired repulsion, *and at the same time we get mitotic figures corresponding to the configuration of the lines of magnetic force between opposite poles.* That is, we get a configuration of spindle fibers and astral rays precisely like the actual ones.

The cases of tri- and multi-polar spindles, so difficult to explain on electrostatic grounds present much less difficulty here. If each centrosome were oscillating along a path radial to the common nuclear center and in the same phase, mutual repulsion, combined with the proper configuration of the astral rays, would be obtained.

The movements and configuration of the chromosomes are also better explained on hydrodynamic grounds than by previous assumptions. It is not even necessary to assume that they execute any independent oscillatory or pulsating motions. Bjerknes has shown that bodies suspended within the field of force of oscillating or pulsating bodies are attracted or repelled depending on whether they are lighter or heavier than the surrounding medium. This attraction or repulsion is due to oscillations induced in the suspended bodies by the permanently oscillating or pulsating bodies. The chromosomes, if heavier than water, or the cell fluid in which they are suspended, would be repelled from each centrosome and would come to occupy a position midway between them in the equatorial plate. Moreover, they would not move outward to the boundary of the equatorial plate. Their induced oscillations, though repelling them from each centrosome, would attract them toward each other, and this action would tend to keep them in the observed axial position. If the chromosomes should become lighter than the cell liquid, the repulsion from the centrosomes would change to an attraction. This immediately suggests that it may be simply a change in specific gravity of the chromosomes which causes them to diverge, after splitting, back toward the centrosomes.

It is now of interest to inquire whether this hydrodynamic action at a distance could possibly be strong enough to account for the actual movements of the centrosomes. It is, of course, almost

impossible to decide this by calculation, in the present state of our knowledge, but if we assume that the centrosomes are smooth, hard spheres and that the cell fluid is homogeneous and as mobile as water, it is not difficult to calculate how vigorously they must oscillate or pulsate in order that they shall move apart with the observed velocity.⁷ Taking the radius as 0.0002 cm., the distance apart as 0.003 cm., the time required for this maximum separation of the centrosomes as fifteen minutes; if the amplitude of oscillation equaled two diameters, the frequency required would be 2000 oscillations per second; if the amplitude were eight diameters, the required frequency would be 100 oscillations per second. With similar dimensions, if the centrosome *pulsated* so that its greatest volume were three times its least volume, a frequency of some 130 pulsations per second would be required.⁸

These frequencies are greater than one would expect. They do not, however, involve any great linear velocities, for the dimensions of the particles are very small. Thus the frequency of 2000 oscillations to the second only means a linear velocity of the centro-

⁷ The formula of Stokes

$$F = 6 \pi \mu v r$$

(Brit. Assoc. Report, p. 445, 1887) applying to the motion of spheres through viscous media was used to determine the force needed to give the centrosomes the observed velocity. To find the needful frequency of oscillation this was equated to the expression

$$\pm 6 \left(\frac{S^2}{4 \pi d^4} \right)$$

derived by Bjerknes for the attraction or repulsion between oscillating spheres.

Similarly, to find the needful frequency of *pulsation* it was equated to the expression

$$8/9 \frac{\pi^3 r^6}{d^2} (a^3 - 1)^2 p^2$$

also based on a formula derived by Bjerknes. In all of these expressions r represents the radius, d the distance between the centrosomes, μ the coefficient of viscosity of the medium (water), p the frequency, S the "action moment," v the velocity, and a the ratio between the mean and the maximum radius of the pulsating sphere.

⁸ It might also be pointed out here that similar calculations on the hypothesis of electrostatic action show that, if the capacity of the centrosomes is simply that of conducting spheres in an isolating medium, a potential difference of nearly two volts would be required; if the capacity is that of spheres surrounded by a "Helmholtz double layer," a potential difference of only a few thousandths of a volt would be necessary.

some of 2 cm. per second. It is, of course, almost impossible to say what effect a viscous, heterogeneous field would have upon the calculation, so we are obliged to leave the quantitative side of the question as quite unsettled.

Besides the oscillatory currents produced by pulsating and oscillating bodies, Bjerknes has shown the existence of steady currents in the fluid medium toward and away from the centers of motion. Similar currents have been observed in dividing cells, particularly centripetal currents between the astral centers. It does not, however, seem wise to treat this or similarly less pronounced phenomena in our present state of ignorance.

The assumption of a pulsating centrosome or centrosphere is by no means an impossible one. The assumption of an oscillating centrosome is not even improbable. The assumption of synchronous pulsations or oscillations involves no mysterious synchronizing mechanism. Random oscillations or pulsations would certainly tend to become synchronous by mutual interaction, while after the closed spindle fibers had formed, whatever their nature may be, any other rate of oscillation would be very improbable.

The fact that such oscillation or pulsation have not been described is not conclusive.

Our knowledge of the subject is based almost wholly on dead material, and moreover the oscillations and pulsations may be very rapid and small.

In conclusion, I would again like to emphasize that the above is nothing but an *ad hoc* constructed hypothesis and intrinsically therefore only of hypothetical value. If, however, it calls attention to a little known phenomenon or stimulates fresh observation it will have served its purpose.

THE REACTIONS OF PLANARIANS TO LIGHT

BY

HERBERT EUGENE WALTER¹

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I INTRODUCTION

Light is one of the physical factors which influence the behavior of organisms. The great majority of living things are normally subjected to regular periodic changes in the amount of light to which they are exposed during the alternation of day and night. In addition to these constant periodic changes, there are innumerable irregular gradations in both the intensity and the character of the light naturally acting upon any organism. An agent of such wide range and almost universal influence as light ought, therefore, when properly analyzed, to prove of material service in interpreting the behavior of animals and plants. The dependence upon light of animals provided with organs of sight, is self evident. The direct bearing, too, of light upon chlorophyllaceous plants in the manufacture of their food substance, is plain. But how far light plays a direct part in the life of non-photosynthetic plants and of animals which cannot "see," is less clear.

Although possessing eyes, it is very probable that planarians are unable to see in the sense of distinguishing shapes, and it is questionable how far they can distinguish between even large regions of different light intensity.

The object of the following paper is to examine the relation of light to animal behavior as applied to certain planarians.

II HISTORICAL

Our knowledge of planarians, as of most other animals, has passed through certain historical phases, during which emphasis has been laid first upon taxonomy and anatomy and latterly upon embryology and zoögeography. The results of these various forms of investigation are highly important since they make the foundation for all future work upon this group of animals. They have, however, only an indirect interest in the present connection and do not, therefore, require review.

Perhaps the most modern advance in our knowledge of planarians is represented by the school which treats of them as living objects whose individual behavior is to be intimately correlated both with their structure and environment. The most noteworthy contribution from this standpoint has been made by Pearl ('03), who has analyzed in considerable detail the reactions of fresh-water planarians (notably *Planaria maculata*, *Planaria dorotocephala* and *Dendrocœlum lacteum*) to various stimuli. He has not, however, discussed the effects of light except incidentally.

The earliest reference to the relation of planarians to light is by Dalyell ('14). In his interesting volume on planarians a great number of keen observations upon the general habits and structure of planarians are made, which have since been confirmed, together with certain statements which have not fared as well with the advance of scientific knowledge.

He makes the statement ('14, p. 9) that "most planariæ *court the light indeed*;¹ but *P. flexilis* rather inclines to shun it, less, we may conjecture, from being warned of its presence by the specks or eyes, than from some disagreeable sensation produced on the body." Again, referring to *P. felina* ('14, p. 46), "This planaria, like the rest of its genus, is powerfully excited to motion by the presence of light. If a number be confined in a glass vessel, the whole assemble in a quiescent state, *on the side next the light*.¹ It is a little surprising that Dalyell should have received the impression that the majority of planarians "court the light," since he clearly points out the nocturnal habits of these worms. He

¹The italics are mine.

doubts whether the eyes are of service in finding food and says of worms under aquarium conditions ('14, p. 107), "If remaining a considerable time unchanged, the planariæ decrease more rapidly, they become languid, scarcely moving either by the influence of the light or heat, and at last adhere entirely to the side of the containing vessel, where they perish."

Dugès ('28) observed that when light is concentrated by means of a lens upon either *Dendrocœlum* (?) or *Planaria*, movement results which is most pronounced when directed toward the anterior end of the worm. He tested the effects of direct sunlight and of diffuse daylight as well as of candle-light, and concluded that the response increases with the intensity of the light. The non-dioptic character of the eyes he has described remarkably well for one working so long before the days of the microtome, and his conclusion, already suggested by Dalyell and later confirmed by Kennel ('88), and others, that the eyes play no part in the finding of food, is noteworthy. He also notes that planarians seek the dark.

Dalyell ('53, p. 99), in a later volume says, "On April 29 I procured a fine specimen of *Planaria cornuta*, which spawned soon afterward. The spawn had been breaking up for two or three days preceding May 24, when multitudes of extremely minute yellow specks were seen swimming in the water. Their motion was sufficiently active, without being very quick; it was pursued in all directions and the spawn being contained in a small cylindrical jar, the specks crowded to the sides next the light whereon numbers remained almost stationary." Again ('53, p. 104), "When withdrawn from the dark the young *Planariæ* rose in great numbers toward the surface of the water, congregating on the sides next the light." It is extremely doubtful whether the organisms here described were really young planarians. It is more likely that they were the young of some other aquatic animal. Dalyell correctly describes *Planaria lactea* (*Dendrocœlum lacteum*?) as being nocturnal. He observed that numbers of this species, beginning activity in the evening, rose on the sides of the jar, although many had descended again by morning.

More recently attention has been specifically directed to the

light relations of planarians in various papers by Loeb, whose important contribution in 1890, "Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen," paved the way in general for all work on this subject. He found ('93b, p. 101) that *Planaria torva* is not "heliotropic" in the strict sense, but rather "unterschiedsempfindlich," that is to say, it did not always move away from the source of the light in the direction of the rays and remain as far removed as possible, but moved about more or less at random, coming to rest in some area of lessened light intensity.

In a later paper Loeb ('94, p. 255) states that when planarians are suddenly brought into the light they begin to move, an increase in light intensity leading to activity and conversely a decrease, to rest. The grounds for this conclusion are not made clear. He further confirms the view that planarians are active at night, coming to rest in situations of lowered light intensity in daytime.

In further experiments *P. torva* when decapitated was found to react to light precisely as normal worms do with the difference that the reaction required more time. *Thysanozoön brochii*, a polyclad, on the other hand, lost its power to respond to light when the eyes and brain were amputated, from which Loeb draws the conclusion that animals which are closely related morphologically may exhibit wide physiological differences.

Hesse ('97), in his classic study of the anatomy of the turbellarian eye, mentions some experiments and observations on the behavior of planarians and in addition makes valuable contributions to the morphological basis of light reactions. He observed that planarians become active at twilight and he also experimented upon decapitated worms, apparently without being aware of the previous work by Loeb, with whose results his own agreed. He found, as Loeb did, that worms deprived of eyes finally come to rest in areas of lesser intensity much as do normal worms but after a longer time. Hesse found, too, that *Dendrocœlum lacteum* came to rest in the dark 119 times out of a possible 120, whereas with *Euplanaria* (*Planaria*) *gonocephala* the same result was effected in only 88 out of 120 times, notwithstanding the fact that the latter has more highly developed eyes than *Dendrocœlum*.

This led him to state ('97, p. 552) that "die Stärke der Reaction auf Lichtwirkung nicht der Stärke der Lichtwahrnehmung entspricht," and he ascribes this difference in behavior to a difference in the "Gefühlston" of the two species. Among other observations described by Hesse, the two following are of importance in this connection, namely, that a sudden introduction of light caused an almost immediate *turning away* on the part of the worm, and, that worms with eyes could not be made to remain in the light when escape was possible. In his opinion this apparent perception on the part of the worm is due not to the animal's ability to distinguish light but rather to unpleasant chemical reactions set up within the organism as the result of light stimulation. And, lastly, Hesse showed that the general position of the eyes of a planarian, together with the arrangement of their sensory portion, partly enclosed as it is within pigment cups, affords a device whereby the worm can be oriented to light. By means of this simple apparatus it receives a localized stimulus, which enables it to distinguish the direction from which the light comes. If light, striking the eye of a worm, fell upon sense cells which were unscreened in any way by pigment, there would result a general stimulation without localization of the stimulus and consequently orientation could not be effected.

Parker and Burnett ('00) sought, by quantitative methods and with more accuracy than Loeb or Hesse, to establish the part played by the eyes in light responses of planarians. They came to the same general conclusion as these authors since they found that *Planaria gonocephala* without eyes reacts to light essentially as normal animals do, except that the reaction time is somewhat longer. They also showed that worms when pointed toward the source of light travel at a slower rate than when headed in the opposite direction. With regard to the mechanism of the light response they say ('00, p. 383): "We have seen nothing in our experiments that supports the opinion suggested by Hesse (p. 551) that reactions such as we have described are due to the direct influence of light on the internal parts of the planarians, and we are more inclined to the view that these reactions are initiated by the effect of light on the integument of the animal, *i. e.*, are

due to what Graber ('83, p. 229) has called a 'dermatropic' function."

Bardeen ('01a, p. 13), speaking of *Planaria maculata*, states that "susceptibility to light is apt to become lost if worms are kept in captivity," and he notes the fact, already brought out by Chickoff ('92), that pigment becomes reduced in sunlight. Hesse had previously emphasized the point that the pigment of the eye of any organism has in itself primarily nothing whatever to do with light perception. Bardeen further found that small pieces of planarians capable of locomotion will respond to light in the same way as uninjured animals, and he notes ('01a, p. 13), that the worms seem "to move about more by night than by day." In a later paper ('01b) he speaks of the fact that when a dish containing planarians is brought into light the worms are commonly roused to activity, although how far such activity is due to light and how far to mechanical disturbances he does not make clear.

Lillie ('01), experimenting upon the regeneration of *Dendrocœlum lacteum*, discovered that posterior headless parts fail to give the typical reaction to light and are incapable of regeneration. He draws the conclusion ('01, p. 132) that "any symmetrical piece of *Dendrocœlum* capable of regeneration tends to come to rest in the shaded part of the dish precisely like a normal individual" and that parts incapable of regenerating "also become incapable, after a day or two, of performing the usual reactions to light." These results on *Dendrocœlum*, it will be seen, are similar to those Loeb obtained in experiments upon the polyclad *Thysanozoön*.

Curtis ('02) reports from laboratory observations 42 cases of fission in *Planaria maculata*, of which number 39 occurred between 10 p. m. and 6 a. m. He adds, however ('02, p. 524), that "this did not seem due to the amount of light to which the animals were subjected during the day, for some of the dishes were so shaded that there was practically no light, day or night, except when they were being examined, and the division was the same in these as in others which were exposed to full daylight." A case of division in *Bipalium* also occurring by night is described by Lehnert ('91).

In a contribution to the geographical distribution of *Planaria*

gonocephala, *P. cornuta* and *P. alpina*,² Voigt ('04) incidentally refers to the manner in which these animals come to rest in the darkest part of a dish. He affirms that when an aquarium is suddenly lighted at night only those that are hungry, *i. e.*, those with comparatively empty digestive tracts, are found in motion, and he notes that in certain conditions worms may remain quiescent for weeks. The statement made earlier by Dugès, that the eyes of planarians play no part in finding food, Voigt confirms. These organs he explains are an aid in distinguishing differences in light intensity as well as the direction from which light comes but are entirely incapable, owing to the simplicity of their structure, of discerning the form of objects. In his opinion worms crawl into hollow stems and similar sheltered places to escape light rather than for warmth, as Wilhelmi ('04) suggests. Neither author, apparently, considers the possible part played by thigmotaxis under such circumstances. Of the delicacy with which worms react to light Voigt says ('04, p. 173): "Die Empfindlichkeit der Planariden gegen plötzliche Belichtung tritt so scharf hervor, das sie für den Unterricht eines der anschaulichsten Beispiele zur Demonstration der Lichtflucht bei niederen Tiere darbieten." Notwithstanding this high degree of sensitiveness to light, he finds that the worms when seeking their food leave the shade and come out even into direct sunlight. And, finally, concerning the bearing which light has on the problem of distribution, he concludes ('04, p. 175): "Auf die Verbreitung im Allgemeinen hat die Belichtung der Bäche wenig Einfluss, da sich in der Regel genug dunkle Schlupfwinkel finden, in denen sich die Tiere verbergen können."

Darwin ('44, p. 242) observed that *land planarians*, "especially *Planaria tasmania*, had an immediate apprehension and dislike of light, which they showed by crawling, when the lid of the box was taken off, to the under side of pieces of rotten wood," and in his enumeration of the places where various species of land planarians were found, their avoidance of light is plainly shown.

A note by Leidy ('58) refers to finding *Rhynchodemus sylvaticus* crawling about on fences frequently at night, but rarely by day.

² *P. alpina* = *P. torva* according to Borelli ('93).

Moseley ('74, p. 111) states that "land planarians are probably all of them nocturnal in habit." Speaking of the Ceylon land planarians in particular he says: "They are found in dark places, such as under large fallen leaves, and in confinement they coil themselves up away from light." He mentions also the fact that *Planaria torva* and *Dendrocœlum lacteum* choose the dark side of the vessel in which they are contained.

As has already been mentioned, Lehnert ('91) found *Bipalium kewense* undergoing fission in the dark. Both *Bipalium* and *Geodesmus*, he says, seek continually to hide in shadowy places avoiding even diffuse daylight. Concerning the degree of light perception possessed by planarians, he offers the opinion ('91, p. 326) that "*Bipalium* scheint mit seinem Augen die Umrisse von Gegenständen in Lichte wahrnehmen zu können."

Hogg ('97) notes that *Bipalium* is nocturnal in habit, remaining sluggish during the day.

Only incidental references to the *polyclads* are found bearing upon the question of light reactions, as for example this sentence, which occurs in Lang's exhaustive monograph ('84, p. 641), "Die meisten Arten scheuen das directe Sonnenlicht." The behavior of *Thysanozoön* with reference to light has already been mentioned (Loeb, '94).

Concerning the light reactions of the rhabdocœles, especially certain green forms in which the green cells are probably symbiotic, a considerable literature may be found. The principal papers relating to these forms are as follows: On *Convoluta schultzei*, by Geddes ('79), Barthélemy ('84) and Delage ('86); on *Convoluta roscoffensis*, by Haberlandt ('91), Bohn ('03a, '03b, '03c), Gamble and Keeble ('03) and Fühner ('06). *Vortex viridis* and *Mesostomum viridatum* (?) are discussed by Schultze ('51), von Graff ('84) and Sekera ('03). A résumé of these papers is, however, out of place here, since the presence of green cells in the organisms involves an entirely different problem from that which is under consideration.

The foregoing historical sketch furnishes the basis of the following general summary of facts which have thus been established with more or less certainty regarding the reactions of planarians to light.

- 1 Planarians are nocturnal, seeking the dark when exposed to light.
- 2 The eyes are useless in finding food.
- 3 The anterior end of the body is the part most responsive to light
- 4 Decapitated worms act normally except for a slower reaction time.
- 5 Orientation to light depends largely upon the character of the pigment cups of the eyes.
- 6 The relative energy of the response is dependent upon the intensity of the light.
- 7 Pigment is reduced in sunlight.
- 8 Pieces of worms which are large enough to move or regenerate react to light.
- 9 Fission *may* occur more readily in the dark.
- 10 Different species respond differently to light.
- 11 Light reactions diminish during "captivity."
- 12 Planarians are "unterschiedsempfindlich" instead of "heliotropic."

III MATERIAL

The species principally used in the following investigations were *Planaria maculata* Leidy; *Planaria gonocephala* Dugès; *Phagocata gracilis* Leidy; *Dendrocœlum lacteum* Oersted; and *Bdelloura candida* Giard, all of which are inhabitants of fresh water except *Bdelloura*, a salt-water species, found living semi-parasitically on the horseshoe crab (*Limulus polyphemus*). Some observations also were made upon a cave planarian, that as yet has not been identified but which may belong to the genus *Phagocata*. This interesting worm was kindly placed at my disposal by Dr. A. M. Banta.

At any season of the year an ample supply of fresh material was easily obtained except in midwinter, when it was necessary to cut through the ice and dredge up from the bottom water-weeds to which the worms cling.

The source of supply for *Planaria gonocephala* was a small pond to the west of Fresh Pond in Cambridge, Mass., while *Pla-*

naria maculata, *Dendrocœlum* and *Phagocata* were chiefly obtained from a pond at Falmouth, Mass., where they are especially abundant. Twice, through the kindness of Professor Parker, aquaria were generously stocked with *Dendrocœlum*, from a spring on Mount Monadnock, N. H. *Bdelloura* was obtained from Wood's Hole, Mass., during the summer from freshly caught horseshoe crabs and, later in the year, from specimens kept in captivity.

The setting-up of balanced aquaria in which planarians would thrive did not prove to be a difficult matter. The following method, based largely upon suggestions by Wilhelmi ('04), was used. Jars were filled to the depth of two or three inches with cinders, dirt and dead leaves, over which was spread an equally deep layer of clean sand. Clear water was then poured into the remaining space and the whole allowed to settle, after which a few such plants as *Anacharis* or *Myriophyllum*, with whatever microscopic life might adhere to them, were added, together with a handful of large pebbles to diversify the bottom. The jars were kept covered from dust in a cool place and occasionally a crushed snail was dropped into each one to supply the worms with food.

Planarians require pure water. Whenever for any reason the water in which they are kept becomes foul they will desert their places of concealment and crawl up the sides of the jar, while water that has been standing in lead or iron pipes quickly causes them to disintegrate. Rainwater or water taken directly from some natural source, gives better results than that which has been conveyed through pipes. Naturally the least chemical disturbance takes place when the worms are kept in water dipped up at the time and place of their capture.

Planarians will live without being fed for over three months when isolated in jars containing nothing except pure water, but meanwhile they decrease regularly in size. It seems to be impossible to "starve" them in the sense in which higher animals may be forced to die from lack of food leaving behind a dead body. These worms instead simply consume their own substance almost to the vanishing point.

During a part of the summer of 1905 observations and experiments were carried on at the laboratory of the U. S. Fisheries

Bureau at Wood's Hole, Mass., and I wish here to express my thanks to the director, Dr. F. B. Sumner, as well as to others in authority there, for their uniform courtesies. The bulk of the investigation, however, was made at Harvard University. I am deeply indebted to Professor Mark for the privilege of having a place in his laboratory and particularly to Prof. G. H. Parker, under whose immediate direction the work was done and whose daily counsels and generous suggestions were indispensable.

IV CRITERIA FOR MEASURING BEHAVIOR

Both the form and the structure of an animal set a limit to the character and degree of its movements, which no combination of stimuli, external or internal, can force it to overstep. In estimating the influence of light upon planarians, therefore, it is necessary to know not only the normal behavior of the worms but also the possible range of their reactions under any circumstances. For example, the ordinary gliding locomotion of planarians is accomplished by means of cilia beating in a mucus track and augmented by muscular contraction. It is physically impossible for this sort of locomotion, even under the most favorable conditions, to exceed a certain rate. By the use of excessive stimuli, however, a worm may be forced to abandon this accustomed gliding for a somewhat faster method of progression known as "crawling" or "humping," in which the muscles are used more than the cilia. But when this is done the limit of possible rate of locomotion has been reached, at least for fresh water planarians, which cannot be urged to abandon entirely contact with some support and to swim freely in water, although the marine form, *Bdelloura*, does have this addition to its repertory of behavior.

The following observations may illustrate more specifically what is meant by range of behavior. *Planaria maculata*, when gliding on the bottom of a dish, was lightly touched on the anterior end with a hair mounted on a glass rod. During one hundred trials of this kind eight different responses resulted, which may be indicated as follows:

	Times
1 Contracted, and turned aside.....	32
2 Contracted, lifted up the anterior end, and turned aside.....	27
3 Contracted, lifted up the anterior end and went straight forward.....	17
4 Contracted momentarily and then went straight ahead.....	5
5 Did not contract but turned aside.....	2
6 Did not contract but lifted up the anterior end and turned aside.....	7
7 Did not contract but lifted up the anterior end and went straight forward.....	9
8 Did not contract but went straight ahead.....	1
Total.....	100

Animals which, like planarians, present a limited range of behavior are, therefore, more favorable subjects for experimentation than higher forms whose structural complexity increases their possible responses, making in consequence the analysis of cause and effect in their activities more difficult. It is evidently desirable, then, to have as many different ways for measuring behavior as possible, in order not to state these responses loosely from general impressions but in quantitative terms. The principal criteria of planarian reactions to light used in this study, follow:

1 *Rate of Locomotion.* Since the entire range of possible rates of locomotion depends upon the structure of the worm and is not very great, slight differences become significant.

2 *Amount and Character of Turning,* that is, whether persistent or irregular, decided or vague, clockwise or contra-clockwise.

3 *Change of Course.* A change in the character, but not necessarily in the direction, of the course is referred to here. "Circus movements," for example, would not be included under this heading because the curving path in such cases, although constantly changing in direction, does not change in character. Tangents to a circle, however, as well as angular and abrupt deviations from a straight line may properly be regarded as changes of course.

4 *Interval of Response.* The apparent effect of light is not immediate in all cases, therefore, the time elapsing between the application of the stimulus and the response to it is a valuable measure of reaction.

5 *Degree of Wandering.* In a sense the degree of wandering shown by a worm is a measure of its indifference to the stimuli acting upon it. It must be noted, however, that apparent indif-

ference may sometimes be due to a balance of opposing stimuli, in which case wandering or aimlessness is not a true measure of the effect of any single stimulus.

6 *Orientation.* This is a measure of behavior with reference to the source of the light. It is expressed by the degree of positiveness or negativeness which the worm exhibits.

7 *Duration of Movement.* The time it takes a worm to tire out when subjected to certain stimuli or, in other words, a measure of fatigue.

8 *Effect of Repetition.* A measure of response is here referred to which may be expressed quantitatively in units of time or qualitatively in manner of behavior.

9 *Wigwag Movements.* These are waving movements of the anterior end of the planarian, which appear to be a definite attempt on the part of the worm to become adjusted to the stimuli acting upon it.

10 *The Time Required to Leave a Unit Circle.* This is a rather unsatisfactory criterion because it may indicate in some cases a combination of several conditions as, for instance, latency of response, rate of locomotion and degree of wandering.

11 *Manner of Coming to Rest.* Included under this heading are such points as the position assumed, the locality selected, and the abruptness of the act.

Naturally some of the foregoing measures of behavior will be seen to have more application and value than others in the following study.

V OBSERVATIONS

I PHOTOKINESIS

The term photokinesis was introduced by Engelmann ('83) to denote the activities which are induced solely by the intensity of light when the directive or orienting factor has been eliminated.

In this section will be considered, (A) the behavior of planarians in the absence of light; (B) their behavior in different intensities of non-directive light, and (C) the effect of abrupt changes, both in time and space, in the intensity of non-directive light.

A Behavior in Dark

Darkness may be called the zero point in the scale of light intensities. That light is not essential to the activity of planarians is shown by their performances in its absence, as is demonstrated by the following facts.

Rate of Locomotion. The average rate of ten individuals of *Planaria gonocephala* was found to be 0.50 mm. per second in the dark while the same ten worms, subjected to a light from above of 38 c.m.,³ with all the other conditions unchanged, averaged 0.82 mm. per second.

Again, ten worms of the same species were allowed to travel in the dark ten minutes in one set of experiments and six minutes in another, when their average rates were found to be 0.42 and 0.57 mm. per second, respectively.

The method devised for obtaining the above records, previously used in experiments upon fresh water snails (Walter, '06), although tedious was comparatively accurate. A clean glass plate was submerged in a dish of water and the latter placed in a light-proof receptacle. A single worm was then allowed to travel on this glass for a unit of time, after which the plate was removed and "developed" by pouring over it powdered carmine shaken up in water. A sufficient number of the insoluble carmine particles adhered to the mucus-track left on the glass by the gliding worm to make it possible to wipe dry the reverse side of the plate and to trace thereon in ink the exact course taken by the worm. This permanent ink line was then measured by means of a map measurer such as is in common use for measuring sinuous lines.

A series of experiments, to be described more in detail later (Table III, p. 57), forms a basis of comparison with the foregoing records in the dark, and further shows that there is an increase in the rate of locomotion in the light.

Ten worms, subjected to various intensities of light projected from above and ranging from less than one to several hundred candle meters, showed rates which in all cases were greater than the rate traveled in the dark.

³ The abbreviation c.m. is used to denote candle meters.

Turning. That planarians do more turning in the dark than they do in various intensities of non-directive light is apparent from the following table of percentages.

TABLE I
Per cent of turnings of *Planaria gonocephala* in the dark and in various intensities of light

Light in candle meters.....	0 (dark)	0.94	11	39	78	126	155	217	431	Av. of all intensities
Per cent of turning.....	87	76	66	69	81	67	75	77	65	72
Per cent of straight paths.....	13	24	34	31	19	33	25	23	35	28
Number of observations.....	71	79	67	85	57	62	67	57	58	

Furthermore, out of a total of 46 cases of turnings made by different individuals of *Planaria gonocephala* in the dark 23 were clockwise and 23 contra-clockwise. This perfect balance in behavior did not recur when the same worms performed turning evolutions in the light.

Change of Course. As to what constitutes "definite" and what "indefinite" changes of path, an S-shaped course is to be regarded as an indefinite aimless wandering, whereas angles in a straight path or tangents in a curving path are classed as definite responses because they are what would normally occur if some directive stimulus were interposed. It was found that *P. gonocephala* made *indefinite* changes in its course more frequently in the dark than in any series of light intensities to which it was subjected for an equal length of time. On the other hand *definite* changes occurred oftener in the light, although the factor of directive light had been excluded.

Table II summarizes 350 records on 10 different worms with the results reduced to percentages.

It will be seen that the per cent of S-shaped ("indefinite") paths in the dark decidedly eclipses that which was made in any intensity of light, while the per cent of angular and tangential paths ("definite") laid in the dark is exceeded in every instance by that made in any intensity of light with one exception, viz: 11 c.m., which, however, is not sufficient to change the average result.

Summary. Planarians move about in the dark but at a slower rate than in non-directive light whatever the intensity. They

turn more in the dark than in the light, going clockwise or counter-clockwise with equal readiness. Finally, they make more indefinite changes in their paths in the dark, but fewer definite changes than in the light.

TABLE II

Percentage of definite and indefinite changes in the character of the course in dark and in light of different intensities

		Details of the several intensities employed								
		Average o for all in- (dark) tensities	0.94	11	37	78	126	155	217	431
Light in candle meters.....										
Definite changes (angular or tangential changes), per cent.....	18	28.5	30	15	27	32	35	21	32	40
Indefinite changes (S-shaped paths), per cent.....	47	23	21	30	23	20	26	34	20	99
No change in character of course, per cent	35	48.5	49	54	50	48	39	45	48	51
Number of observations.....	34	316	49	35	48	37	31	48	35	33

B Non-Directive Light

a Apparatus

To test the effect of purely non-directive light, it is of course necessary to eliminate the possible influence of directive light. This may be done by projecting the light upon the moving worms in such a way that they are unable to go either toward or away from the source of the light. Whatever effect is obtained under such circumstances must be ascribed to the non-directive power of light.

The elimination of the directive influence of light can be accomplished by means of various devices. (1) The light may be made to fall vertically from above upon a horizontal field; (2) it may be reflected vertically from below so as to pass through a transparent field at right angles to the plane of the field; (3) methods 1 and 2 may be combined. The apparatus finally used in the majority of experiments with non-directive light, was based upon the method first mentioned.

Fig. 1 shows a diagrammatic vertical section of this apparatus. The light (*A*), an incandescent electric lamp, was mounted in a black sheet-iron hood (*B*) to prevent the escape of any lateral light. This hood was suspended from the ceiling of the dark room where the experiments were carried on and was arranged so that it could be easily raised or lowered, thus changing the height and consequently the intensity of the light with reference to any fixed point below. In the hood, beneath the light, was supported

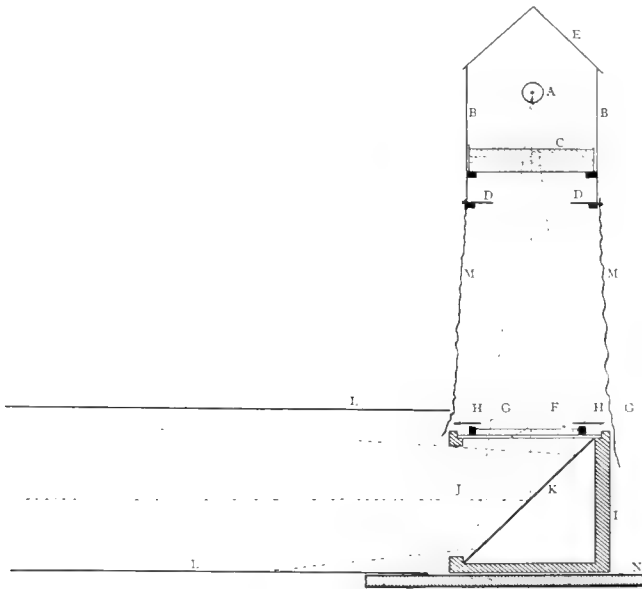


Fig. 1 *A*, light; *B*, walls of hood; *C*, heat screen; *DD*, diaphragm; *E*, roof of hood; *F*, plate-glass floor of aquarium; *G*, paraffine wall of aquarium; *HH*, diaphragm to cut off light reflections from paraffine wall; *I*, wall of reflector box; *J*, open side of reflector box; *K*, mirror; *L*, walls of tunnel; *MM*, black draperies; *N*, table.

a flat-bottomed, clear-glass dish (*C*) containing distilled water to a depth of about three centimeters. The heat screen thus obtained effectually filtered out the heat rays, allowing only the light rays to pass through. A few inches under the heat screen was inserted a diaphragm (*D*), painted black, the purpose of which was to aid in cutting out side reflections besides allowing only a central column of light to escape below. A black sheet-iron roof (*E*) con-

fined the upward rays to reflections within the hood itself, at the same time permitting the escape of heated air. On a table directly under the suspended light lay a horizontal sheet of plate glass (*F*,) affixed to the upper surface of which was a circular ring (*G*) made of a mixture of paraffine and lampblack. There was thus formed a circular water-tight aquarium twenty centimeters in diameter and two centimeters deep, in which the worms could be observed. On the top of this circular ring rested a black diaphragm (*H*), the aperture of which was sufficiently small to exclude any side reflections which might come from the black paraffine wall.

The aquarium, it must be explained, did not rest directly on the table but was mounted as the cover of a box (*I*), the interior of which had been rendered largely free from reflecting surfaces by the use of black camera-paint. One side of the box was removed and, facing the opening thus made, a mirror (*K*) was placed at an inclination of 45° with the horizon. The end of a square tunnel (*L*), ten feet long and made of black cloth stretched upon a framework of wood, fitted close up to this opening. Suspended from the lower edge of the hood and surrounding the aquarium were adjustable black draperies (*M*) designed to shut out possible side light and at the same time to allow a hole for the eye of the observer. It will be seen that all light reaching the aquarium comes from the lamp above by passing through the heat screen.

After illuminating the field of observation the light passes through the glass floor of the aquarium and is reflected by the mirror into the black tunnel. Most of the light is absorbed in the tunnel, only an insignificant minimum being reflected back to the aquarium floor. Otherwise complications in the character and intensity of the light might arise.

By moving the hood (*B*) up and down and by using lamps of different candle powers a variety of intensities was obtained. The lamps used were tested by means of a Lummer-Brodhun photometer, the loss by reflection from the surface of the water both at the heat screen and at the aquarium being reckoned out in determining the different intensities employed.

By simple observation, data for such criteria of behavior as

amount of turning, changes in course, degree of wandering, interval of response and manner of coming to rest, could be obtained in this apparatus with approximate correctness. To determine the rate of locomotion, however, required a device which would measure accurately the distance traveled in a unit of time. The

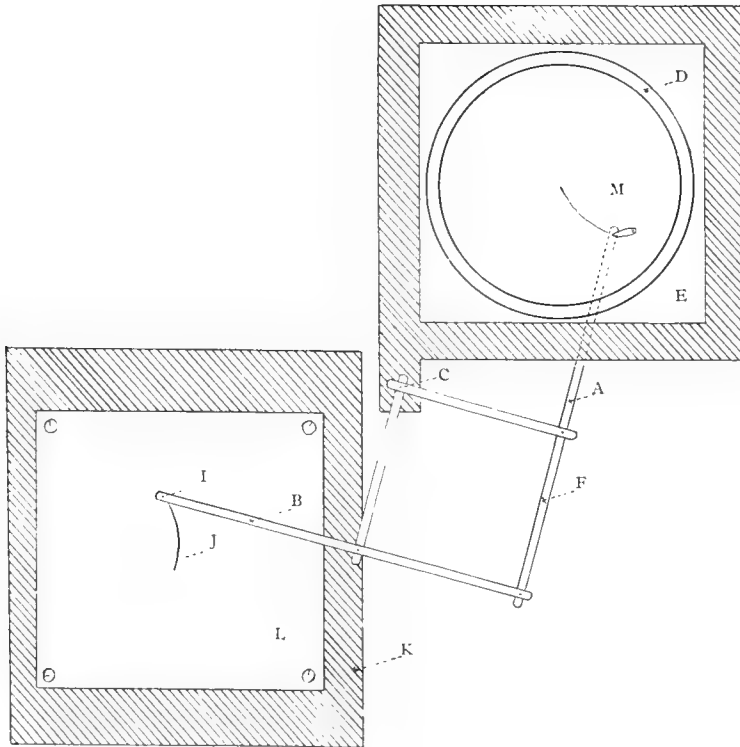


Fig. 2 *ABCF*, pantograph; *C*, fixed point; *D*, paraffine wall of aquarium; *E*, plate glass bottom of aquarium; *F*, place where the arm *A* is grasped by the operator. A style is located at end of arm *A*, in contact with under side of aquarium floor. *I*, style at end of tracing arm *B*, in contact with smoked paper; *J*, beginning of a course traced on the smoked paper; *K*, drawing board for attachment of smoked paper; *L*, sheet of smoked paper fastened to drawing board; *M*, actual course of the worm.

method already mentioned of measuring rate from mucus-tracks developed by means of powdered carmine, proved too tedious and uncertain except for the worm's maneuvers in the dark, when it seemed the only available way.

To avoid the inconveniences of this method an attachment was devised for directly duplicating the path of a worm by means of a style traveling over a sheet of smoked paper. The records thus traced were made permanent by immersing the smoked sheets in a weak solution of resin in alcohol and allowing them to dry, after which the paths could be accurately measured and the rates computed.

The arrangement of this attachment, as seen from above, is shown in Fig. 2. The diaphragm (Fig. 1, *H*) has been removed for the sake of clearness. At the tip of arm *AF* a style directed upward comes in contact with the under surface of the aquarium bottom (Fig. 1, *F*), while at the tip of arm *B* a similar style that is pointed downward traces a line on the sheet of smoked paper *L* at the left. After a little practice it was not difficult to keep the style of arm *A* directly under the posterior end of a gliding worm, thus duplicating its movements with considerable accuracy. The expiration of any time interval can be indicated on the smoked paper record by a crosswise scratch in the path.

Arm *A* was rendered as non-reflecting as possible by black camera paint as well as by being made triangular in cross section with the apex of the triangle upward. Thus whatever rays struck it from above were mostly either absorbed or reflected in a horizontal direction, so that they did not reach the worm under experiment.

b Results

Rate of Locomotion. *Planaria gonocephala* moves somewhat more quickly in non-directive light than it does in dark. Ten apparently normal and representative worms were selected and isolated in individual aquaria. They were kept in the dim light of the dark room in water of the same temperature as that of the experimental aquarium in which they were observed. At the end of thirty-four days of experimentation these worms showed practically the same average rates under the same intensities of light as they did at first. By alternating the individuals these trials were so made that fatigue effects had little part in the results, while the succession of light intensities was varied in such a way

that cumulative effects and the influence of previous exposures were largely avoided.

The results obtained in 259 trials are condensed in Table III.

TABLE III

Rate of locomotion in millimeters per second of Planaria goniocephala in various intensities of non-directive light

Candle meters.....	0	0.94	11	39	78	126	155	217	431
Average mm. per sec.....	0.57	0.66	0.69	0.75	0.64	0.66	0.69	0.70	0.63
Number of records	30	28	30	29	27	30	30	27	28

The mechanical stimulus resulting from the removal of the worms, by means of a camel-hair brush, from their individual aquaria to the observation aquarium was practically the same in all cases as were all the other external stimuli except light. The difference in the rate of locomotion appearing in these averages is, therefore, clearly due to differences in the light intensity employed.

It will be seen also that rate does not increase progressively with intensity. The series of rates and intensities under Table III, if plotted in a frequency curve would give two modes, one at 39 and the other at 217 candle meters, with a slight depression between the two. Still, as has been already pointed out, any intensity of light gives a faster rate than no light at all.

The slowest average rate was made under the highest intensity of light employed. Certain facts to be brought forward later favor the opinion that this was not an accidental result.

Under continuous exposure to one intensity of light the rate of locomotion decreases. The worms seem to "run down" gradually, so that at the end of ten minutes their rate is only about half that during the first minute. Data illustrating this point are given in Table IV.

The rate of locomotion depends not so much upon the intensity of light as upon other factors which tend to produce individual behavior upon the part of each particular worm. Stated in another way, there is greater variation between different individuals in the average rate of their locomotion under all intensities than there

is in the average rate of all individuals collectively under different intensities. The data for this latter point based upon the average rate of ten worms (259 observations) under different intensities has already been given in Table III (p. 57). The extremes in rate there shown are 0.57 mm. per sec. at zero intensity and 0.75

TABLE IV

Average rate of locomotion of Planaria gonocephala in successive minutes of exposure to 39 c.m. of non-directive light

Number of minute.....	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th	11th	12th
No. of records averaged..	17	15	12	7	5	4	4	3	3	2	2	2
Rate in mm. per second..	.63	.625	.565	.55	.53	.55	.375	.39	.39	.29	.25	.29

mm. per sec. at 39 c.m. intensity, which makes a range of 0.18 mm. per sec. When the same data are rearranged to show the average rate for each individual for all intensities, as in Table V, the extremes are 0.49 mm. per sec. and 0.83 mm. per sec. with a range of 0.34 mm. per sec.

In fact the individual behavior of these ten worms, despite their apparent similarity, was sufficiently distinct to allow each one to be thereby identified.

Turning. Attention has already been called to the fact that there is less turning in light of various intensities than in the dark. A return to Table I will make plain that there fails to be any

TABLE V

Average rate of locomotion for each of ten worms (Planaria gonocephala) based on trials with non-directive light of various intensities

Identification number of worm.....	1	2	3	4	5	6	7	8	9	10
Average rate in eight intensities expressed in mm. per sec.....	0.79	0.57	0.68	0.64	0.83	0.70	0.72	0.58	0.49	0.62

definite correlation between the degree of intensity of the light and the amount of turning, although the least turning occurs under the highest intensity. This latter point, however, rests upon a very slight difference and may not be significant. It is nevertheless worth mentioning, since it is in line with the effect of the

highest intensity upon rate, as well as with certain other evidence to be discussed later.

The small excess of clockwise over contra-clockwise turnings is not explainable upon the ground of varying intensities of light. A distribution of the cases under the several intensities of light (Table VI) makes it plain that this peculiarity is due rather to individual causes than to light intensities. Indeed it would be difficult to conceive theoretically how varying intensities of non-directive light could influence a worm in such a way as to affect the direction in which it turns. The natural expectation according to chance would be an equal number of turnings in either direction. The excess of clockwise turns seems, therefore, undoubtedly due to internal causes which render certain worms more liable to go one way than another. In fact, when the records were arranged according to individual behavior it was found that of the ten worms seven averaged a majority of clockwise turns while only three fell in the contra-clockwise column.

TABLE VI

Character of turning of Planaria gonocephala in non-directive light of various intensities

Light in candle meters.....	0	0.94	11	39	78	126	155	217	431	Total
Clockwise turns.....	23	25	21	32	17	17	22	24	22	203
Contra-clockwise turns.....	23	23	17	20	17	18	17	17	14	166

Change of Course. A greater number of "definite" changes occur in the light than in the dark, but fewer "indefinite" changes. This point requires no further exposition as its corollary has already been given.

The behavior of the worm in this respect seems to be more closely correlated with the highest intensity (431 c.m.) than with any other. In the highest intensity employed there are indicated (Table II, p. 52) 40 per cent of definite changes, which is considerably in excess of the percentage of such changes made in any other intensity. On the other hand indefinite, or S-shaped, changes constitute only 9 per cent of all records taken at the highest intensity, which is less than half the number of indefinite paths made in any other intensity.

While the extremes of the series of definite changes indicate a general rise in the percentage of their occurrence with an increase of intensity, and while in the same way the extremes of the series of indefinite changes suggest in general a decrease of frequency with the increase of intensity, it can hardly be maintained that the character of the changes in course is definitely correlated in the majority of cases with changes in intensity.

Degree of Wandering. Wandering is not closely correlated with the intensities of light. In Table VII, which deals with the percentage of straight paths made by *P. gonocephala* under different intensities of non-directive light, this fact is expressed negatively, since it is held that a straight path is a good indication of the absence of aimlessness or wandering and may thus serve as a negative measure of such behavior.

TABLE VII

Percentage of straight paths made by P. gonocephala in the dark and also in non-directive light of different intensities

Light in candle meters.....	0	0.94	11	39	78	126	155	217	431
Percentage of straight paths.....	13	24	34	31	19	33	25	23	35

In this respect again the behavior of the worms under the highest intensity is more pronounced than under any other intensity since the greatest number of straight paths were laid at an intensity of 431 c.m.

Interval of Response. There seems to be some evidence that the interval of time elapsing between the reception of a light stimulus on the part of a worm and its consequent response, may be quite considerable. Three facts were established that may support this conclusion.

First, when two-minute records were made under various intensities, it was found that the worms averaged a faster rate during the second minute of exposure to the light than during the first, in spite of the facts that the mechanical stimulus due to placing the worm in the light machine had a more quickening influence during the first minute and that the fatigue effects were more likely to appear during the second minute. The actual figures

for the above statement, based upon 240 two-minute trials under various intensities, are 0.645 mm. per sec., the average during the first minute, as against 0.713 mm. per sec., the average during the second minute.

Secondly, in these 240 trials, the percentage of turning under all intensities is greater during the first minute than during the second, being 87 per cent and 57 per cent, respectively. This result may possibly be conceived to be due to a greater steadying influence of the light during the second minute than during the first and to a consequent greater turning than during the first minute. But on the other hand a similar decrease of turning, although not so pronounced, took place during the second minute when the worms were in the dark. It must be admitted, therefore, that the fact of less turning during the second minute may have nothing to do with the interval of response.

Thirdly, on several occasions a notable piece of behavior was observed, which may have a bearing on the interval of response. The phenomenon in question always occurred in connection with a modification of the experimental field within the light machine to be more fully described later. Briefly this modification consisted in making a field of two distinct intensities of light, the latter being projected vertically from above in such a way that a sharp line of demarkation formed a boundary between the two areas. Ordinarily when the worms reached this boundary line as they glided from one intensity to another, they responded promptly to the stimulus caused by the change of intensity. Several times, however, they were observed to travel indifferently exactly along this dividing line for a distance of several centimeters with half the body in one intensity and half in the other. This curious fact lends itself to various interpretations, one of which is that the response to a new intensity may not be, in all cases, immediate.

Manner of Coming to Rest. During the experiments made in the non-directive light apparatus previously described, normal worms could never be induced to come to rest in the light. If allowed to remain in the aquarium they would wander about until they reached the shadow under the diaphragm (Fig. 1, *H*),

where they finally stopped, usually in the angle formed by the paraffine wall and the bottom.

Loeb's conclusion ('93b, p. 101) that planarians subjected to directive light come to rest in regions of least intensity, seems therefore to be equally true of planarians in non-directive light.

Summary. In non-directive light *Planaria gonocephala* moves faster, turns less and makes more "definite" but fewer "indefinite" changes than in the dark. Rate of locomotion; amount of turning; changes in the character of the course, as well as the amount of wandering, do not appear to be correlated with varying light intensities, unless in the following instance. Under the highest intensity employed, namely, 431 c.m., occurred the slowest rate; the least turning; the greatest number of "definite" and the fewest "indefinite" responses, together with the straightest paths. The excess of clockwise over contra-clockwise turnings throughout the series of intensities is probably not attributable to light.

Continuous exposure to light results in a decreasing rate of locomotion, although in the second minute of movement as compared with the first an increase in the rate of locomotion takes place, while fewer turnings occur.

Rate of locomotion is less influenced by differences in light intensity than by certain internal factors which go to make up what may be termed the individuality of different worms. Individual worms may sometimes fail to respond for a considerable interval of time to light stimuli that ordinarily produce immediate effects.

Finally, planarians subjected to non-directive light come to rest in regions of lessened light intensity the same as they do in directive light.

C *Abrupt Changes in Intensity*

Abrupt changes in intensity may be of two kinds: either with reference principally to time or to space. First, those changes are abrupt *in time* in which light or dark is suddenly thrown upon the worm, and secondly, those changes are abrupt *in space* in which a moving worm passes immediately from an area of one intensity into a sharply defined area of a different intensity. This topic

will be discussed here only in its relation to non-directive light; the effects of sudden changes in directive light coming more properly in a later section.

a Abrupt Changes of Light Intensity in Time

Whenever worms were left over night in the experimental aquarium completely shut off from light, a large proportion of them would be found at rest in the morning when the light in the hood was again turned on. By removing the diaphragm (Fig. 1, *H*), under the edge of which near the paraffine wall the worms were usually collected, it was possible without any mechanical disturbance to subject resting worms to sudden non-directive light after a prolonged period of complete darkness. This sudden stimulus rarely had an instantaneous effect. The interval of response was often several minutes and frequently non-directive light alone proved insufficient to start the worms into activity.

No sudden increase of intensity ever proved powerful enough to throw a gliding worm into the more rapid method of crawling. Pearl ('03, p. 551) stated the same fact after subjecting planarians to much stronger intensities of light than were employed in the present experiments.

It was found that *P. gonocephala* showed a decided response—either some change in course or a wigwag motion of the anterior end—more frequently when suddenly subjected to dark than to light. By inserting a key into the electric circuit it was possible to control the light in the hood to a fraction of a second. Worms in complete darkness were by this means subjected to various intervals of sudden light and worms in light to intervals of sudden dark, the results being at once noted. While the worms were in the dark their behavior could not, of course, be directly observed, but by watching them closely just before the light was turned off and also the instant it was turned on again there was no great difficulty in determining whether a response had occurred during the interval. The results obtained from nearly a thousand trials are indicated in Table VIII.

It will be seen from this table that there are more responses than failures to respond and that the responses occur more fre-

quently when the worms are suddenly subjected to dark than to light.

It may be further noted that the excess of the responses in the dark over those in the light increases with the interval of exposure, indicating that the worm's adjustment to a change in the light stimulus affecting it is not in all cases immediate.

The effect of previous exposure, whether to several hours of dark or light, is a factor in these results which will be considered more properly later on.

TABLE VIII

Percentage of the responses of P. gonocephala in various intervals of time when suddenly subjected to dark and to light of 39 c.m.

Number of seconds exposed.....	5	10	15	20	25	30	Average
Percentage of responses in light.....	51	59	54	54	48	46	52
Percentage of responses in dark.....	63	66	73	75	71	71	70
Excess of responses in dark.....	12	7	19	21	23	25	18

It should be added that *Bdelloura* gives a remarkable response when enveloped in sudden darkness. It will frequently forsake its attachment under these circumstances and unattached in the water go through violent contortions. This striking response can be called forth by an exceedingly brief interval of dark, namely, the shortest time required to turn the electric light off and on. Nagel ('94, p. 387) speaks of animals thus affected by sudden shadow as "skioptic."

The relation of *Bdelloura* to light falls into a somewhat different category, however, than that of the fresh-water planarians, since *Bdelloura* is positive to light, while fresh-water flat-worms are negative.

b Abrupt Changes of Light Intensity in Space

Several devices were employed to test the behavior of planarians passing abruptly from an area of one intensity of non-directive light into another. The most successful device tried was that in which two lights of different intensities were mounted overhead

in the hood of the apparatus already described in Fig. 1, the mingling of their rays being prevented by the insertion of a vertical diaphragm (Fig. 3, *C*), which extended from the region between the lights down to the surface of the aquarium. In order to place the diaphragm in position it was, of course, necessary to remove the heat screen (Fig. 1, *C*), the presence or absence of which, however, would not have affected the results sought since the water in the aquarium itself was nearly 2 cm. deep and thus

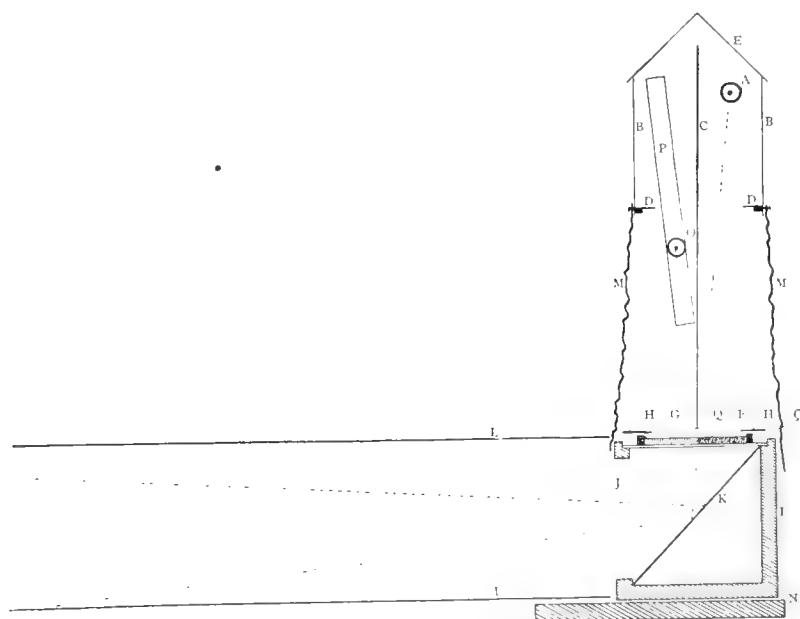


Fig. 3 *A*, stationary light; *B*, sheet iron walls of hood; *C*, vertical diaphragm separating the two lights; *D*, horizontal diaphragm; *E*, sheet iron roof of hood; *F*, plate glass aquarium floor; *G*, paraffine wall of aquarium; *HHH*, diaphragm to shut off reflections from wall of aquarium; *I*, wall of reflector box; *J*, open side of box; *K*, mirror; *L*, black tunnel; *M*, black draperies cutting off side light; *N*, table supporting reflector box and end of tunnel; *O*, movable light; *P*, track for movable light; *Q*, narrow, horizontal diaphragm attached at right angles to the lower side of the diaphragm *C*, in order to prevent the light rays from the two sources of light, *A* and *O*, from overlapping.

constituted an efficient heat screen. By keeping the hood stationary and causing one of the lights (Fig. 3, *O*) to slide up and down at will, it was possible to bring about various contrasts of

intensity in the field below. The complete plan of the apparatus is given in Fig. 3.

The principal variations in the behavior of *Dendrocœlum* and *Phagocata* upon reaching the critical line separating the areas of two intensities are indicated diagrammatically in Fig. 4.

The dotted line represents the boundary separating two areas of different light intensities. The arrows represent the types of paths made by *Dendrocœlum* and *Phagocata*. For the sake of simplicity the worms are represented as going in one direction; that is, into one of the two contrasting intensities, but the same types of paths resulted as well when the opposite direction was taken. The angles made in crossing the critical line were also more varied than those represented in the diagram.

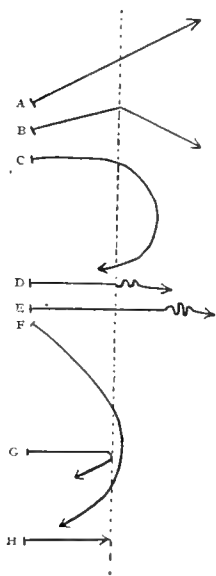


Fig. 4

Type *A* represents a passage without response; *B*, an angular change of course made at the critical line; *C* and *F*, a loop-like return effected after a short excursion into the new intensity, and *G*, a sharp turning aside, while *H* indicates a halt at the critical line, as if a barrier had been encountered. Finally *D* and *E* represent a temporary pause on the part of the worm accompanied by wigwag movements of the anterior end of the body. In the case of *D* the wigwagging is immediate, but *E* typifies a case when there occurred in the response an interval of such a nature that the significant movements were not made until the worm had advanced at least its own length into the new area.

Of these types all, with the exception of *A*, are to be regarded as reactions to differences in intensity encountered. The most questionable are the infrequent types *C* and *F*, which may be otherwise explained as arcs in a curving course which might have occurred in a field of uniform intensity. By far the commonest type was *D*, plainly the least doubtful of the series.

As a result of over 3000 observations on the manner in which the critical line separating the two intensities was passed, three facts become evident. First, responses were considerably more

TABLE IX

Kind and percentage of responses of Dendrocælum and Phagocata in passing from one intensity of non-directive light to another

Character of course	No responses (Type A)	Wigwags (Types D and E)	Turn-backs and full stops (Types G and H)		Loops (Types C and F)	Angular courses (Type B)	Total responses
Going into greater intensity, per cent.	79	11	6		2	2	21
Going into lesser intensity, per cent.	50	36	5		8	1	50
Average responses, per cent.	64.5	23.5	5.5		5.0	1.5	35.5

frequent when the worms were passing into the lesser intensity than they were when entering the greater intensity. Secondly, lack of response is more frequent than a visible response of any kind since 64.5 per cent of the crossings made over the critical line were of the type A. Thirdly, the responses at the critical line were more frequent when the worm was upside down, *i. e.*, moving on the surface film, than when it was on the floor of the aquarium. This latter point was illustrated most fully by Phagocata, which, being an active worm, takes quite readily to the surface film, so that it was possible with this species to get a series of observations in which the behavior when crossing the critical line on the bottom of the aquarium could be compared with that when the same line was encountered at the surface film. Table X contains the results of these observations.

The doubling of responses when the worm is on the surface film is probably not due to an unequal receptivity of light stimulus by the dorsal and ventral surfaces of the planarian as might at first thought seem possible. As will be shown further on, the worm's rate of locomotion on the bottom of the aquarium is nearly the same whether the light comes from below or from above, pro-

vided the amount of light in both cases is equal. Planarians, as Pearl has emphasized, are strongly thigmotactic. Naturally, then, their response to contact is much greater when they are on the glass bottom of the aquarium than when they are suspended on the less resistant surface film. In other words, the less the worm is influenced by the stimulus of contact the freer it is to respond to the stimulus of light.

TABLE X

Percentage of the responses made by Phagocata at the critical line separating two intensities of non-directional light either on the bottom of the aquarium or on the surface film

	Number of observations	No response per cent	Response per cent
On the surface film.....	740	45½	54½
On the bottom.....	1664	76	24
Total.....	2404	60½	39½

Finally, a series of experiments was tried in which the contrast between two intensities was varied by raising or lowering one of the lights in the hood. It was found that the responses made by Phagocata under these circumstances increased with the increase in contrast between the two intensities as shown on the bottom line of Table XI, where these contrasting intensities are expressed in a ratio between the constant light taken as unity and the movable light.

The fact that responses by no means invariably occur when bright light and complete darkness are suddenly substituted for each other (see Table VIII) rendered a further extension of this series unnecessary. The contrasts here used form probably a much greater range of intensity contrasts than the worms ever encounter in nature.

Attention to the details presented in Table XI brings to light the fact that, although the number of responses is correlated in a general way with an increase in the contrast between the two illuminated areas, as shown in the bottom line of the table, yet the percentage of the responses is further influenced by the actual degree of the intensities employed. For example, when the two

TABLE XI

[illegible]

areas of light were respectively 33.16 and 68.18 c.m. the ratio was practically the same as when the two intensities were 16.3 and 33.16 c.m., yet the percentage of responses in the two cases is decidedly different, being 10.5 per cent in the former, and 56 per cent in the latter case. When the lesser of the two lights was 33.16 c.m. there were invariably fewer responses than when the lesser light was 16.3 c.m. The latter intensity is undoubtedly nearer the planarian's optimum intensity, and the apparently inhibitive action of the higher intensities agrees perfectly with certain facts already detailed, as, for instance, that the activities of *Planaria gonocephala* were less pronounced at 431 c.m. than in lesser intensities; and, again, that all planarians show more responses on going into a lesser than when going into a greater intensity.

Attempts were made in some other ways to subject planarians to areas of contrasted intensities and, although the results were less satisfactory in general than those obtained by the method of using two overhead lights of different intensities just described, yet certain facts were brought out that may be worth recording.

In the first of these attempts two concentric rings of white paper, each about two centimeters wide and having between them a space of a couple of centimeters, were fastened to the under surface of the aquarium floor. The white paper thus arranged reflected the light upward and made areas of gradually increased intensity as compared with the remainder of the aquarium floor through which the light passed without reflection. Worms placed in the center of these circles would consequently be obliged to pass from one intensity of light directly to another, whatever the direction of the radius they might be taking. When worms were actually tested, it was found that they exhibited considerable modification in their movements, particularly when approaching the edge of the paper backgrounds.

Owing to the considerable thickness of the plate-glass floor of the aquarium as well as to the fact that white paper is a surface which scatters the light falling upon it, it was found that there was formed, not a sharp line of demarkation between two intensities, but rather a penumbra-like margin of intermediate light. This apparatus was therefore abandoned as unsatisfactory.

The difficulties presented by paper as a reflecting surface largely disappeared by the substitution of a plain mirror in its place, since the surface of a mirror is such that all the light striking it at right angles is reflected at right angles. When, therefore, an unmounted mirror was brought into contact with half of the under surface of the aquarium floor the whole field was thereby divided into two regions sharply separated from each other. Of these one was supplied with light from above only, while the other received the same light plus nearly an equal amount reflected from the mirror below. With the aid of this device an increase of 11 per cent was gained over the responses obtained when white paper instead of a mirror was used as a reflector. Both *Phagocata* and *Dendrocœlum* were tried by this method. In 76 per cent of the trials made, *i. e.*, in 125 cases out of 165, the worms showed a visible modification in their behavior on reaching the boundary of the two areas of light. It was nevertheless decided that this method was an uncertain test of behavior, since the body of the worm, although fairly translucent, would by no means allow all the light that fell upon it to pass through and be reflected, and consequently the difference of the two intensities to which it was being subjected could not be easily estimated.

Summary. When sudden light or dark envelops planarians (*Dendrocœlum*, *Phagocata* and *Planaria*) the response, if any occurs, is often not immediate.

No one of the intensities of light which were employed in these experiments when introduced suddenly was sufficient to make the worms forsake gliding for crawling.

Sudden dark calls out more responses than sudden light, while the number of responses increases with an increasing interval of exposure to the stimulus. *Bdelloura* is decidedly "skioptic."

Worms encountering the edge of a reflecting area which increases the intensity of the light without introducing any other barrier, show a marked degree of response. The percentage of response is considerably larger when a mirror instead of white paper is used to produce the reflecting surface. If worms are allowed to pass from one intensity to another sharply separated from it, their responses are more frequent upon passing into the lesser intensity

than when going into the greater. The average number of failures to respond to these contrasts of intensity reaches about two out of three.

Phagocata, at the critical line separating two contrasting intensities, responds oftener when on the surface film than when gliding over the bottom of the aquarium.

The number of responses increases with the increase in the contrast between the two intensities employed, but the percentage of response is greater, regardless of ratio, when one of the lights is of low intensity (13.6 c.m.) than when both are of higher intensity (33 + c.m.)

2 PHOTOTAXIS

The term "phototaxis" was introduced by Strasburger ('78) in a study of certain swarm-spores, to indicate movements which were parallel with incident light rays. The term has since been extended by several authors to include similar movements on the part of animals. Any organism is said to be positively phototactic when it moves toward the source of light in the direction of the rays and negatively phototactic when it goes in the opposite direction.

The purpose of this section is to consider the phototactic movements of planarians, as distinct from their photokinetic behavior, (A) when the light remains constant, (B) when the light is changed either (a) in intensity or (b) in direction, and (C) when phototaxis is combined with responses of a different kind.

A In Constant Directive Light

Orientation. With the exception of *Bdelloura* all the planarians studied are, under normal conditions, negatively phototactic so far as their first movements in directive light are concerned. To obtain quantitative data for this statement it was necessary to construct an apparatus in which the worms to be tested could be placed quickly and with as little mechanical disturbance as possible in the center of a unit circle with the long axis at right angles to the direction of incident light. The circle was marked off into degrees so that by noting the place at which a worm made its exit a quan-

titative measure of the amount of turning toward or away from the source of the light under the given conditions was obtained.

The apparatus finally utilized for this experiment was based upon a device employed by Parker and Burnett ('00) in testing the relative behavior of normal and eyeless planarians when subjected to directive light. Its arrangement is shown in Fig. 5.

On the top of a table (*A*) in the dark room was placed a rectangular aquarium (*BCDE*), the ends of which (*BE* and *CD*) were

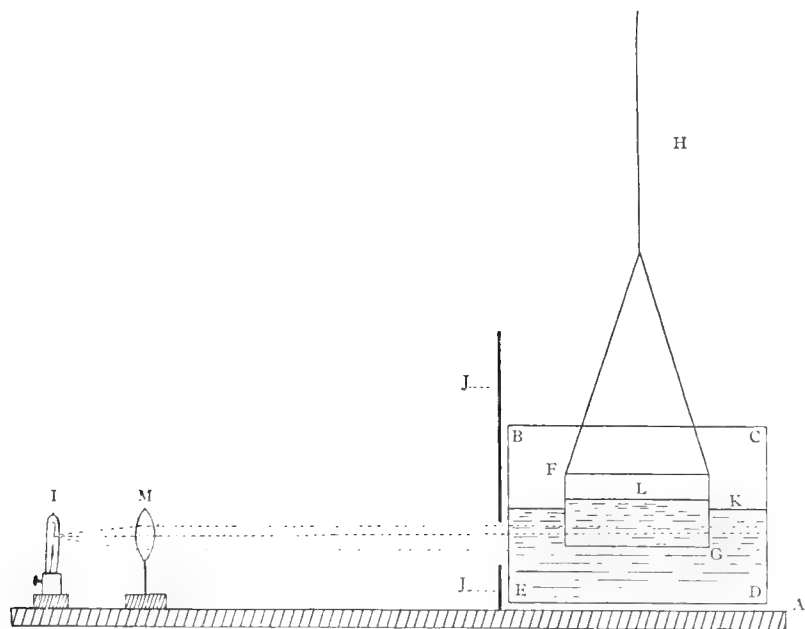


Fig. 5 *A*, Top of table; *BCDE*, rectangular aquarium; *BE*, glass end; *FG*, round swinging aquarium; *H*, copper wire attached to ceiling and supporting the swinging aquarium *FG*; *I*, movable light; *JJ*, diaphragm; *K*, surface of water in outer aquarium; *L*, surface of water in inner aquarium; *M*, lens.

made of glass while the floor and sides were of wood painted with camera-black. Within this aquarium a second cylindrical one (*FG*), made entirely of thin glass and measuring 20 cm. in diameter, was suspended from the ceiling by means of a fine wire (*H*) attached to a swivel to allow turning. On the floor of the outer aquarium and directly beneath the inner one was drawn a circle

10 cm. in diameter and marked off plainly into arcs measuring 5 degrees each. An incandescent lamp (*I*), placed on the table at approximately the height of the inner aquarium floor, could be manipulated at any desired distance, while a diaphragm (*J*) prevented much of the light from reaching either the upper surface of the water contained in the two vessels or the floor of the outer aquarium whence it would be reflected. A biconvex lens was then so interposed as to make the light rays practically parallel upon their emergence from it. Their course through the inner aquarium was kept parallel by means of the medium of water on both its inner and outer sides. A nearly uniform intensity over the entire floor of the swinging aquarium was thus obtained and the objection arising when the inner aquarium is used in air, viz: that it acts as a converging lens, was obviated. Side reflections were eliminated by enclosing the light (*I*), together with the intervening space between it and the diaphragm, with black screens.

When a worm introduced into the inner aquarium began to glide, it could with slight mechanical disturbance be quickly rotated, by means of moving this inner aquarium, into any desired position with reference to the light, and then swung so as to bring its posterior end exactly over the center of the stationary circle below.

Various species of planarians were started in this manner at right angles to the light. Out of 386 cases, 371, or 96 per cent, emerged from the 10 cm. circle at a point farther away from the light than that toward which they were originally directed. This is taken to mean that 96 times out of a hundred the worms were negatively phototactic. If, however, the method of reckoning negativeness employed by Parker and Arkin ('01) on the earthworm is used, the foregoing per cent would be somewhat less. These authors assume ('01, p. 28) that the apparently positive responses of a normally negative animal, such as the earthworm, may be due to causes other than light, in which case an equal number of responses of like nature might be expected to occur on the negative side as well as on the positive. A number equal to the sum of these apparently positive responses should therefore be subtracted from the total of the apparently negative responses

in order to obtain approximately the amount of unquestionable negativeness. By following this method in the case just given, the per cent of negativeness would be 92 instead of 96, but since this method assumes that normally negative worms are never positive, which is contrary to the evidence to be given later, the most accurate estimate of negativeness would probably fall somewhere between these two percentages.

Bdelloura, on the other hand, behaves in the same way only three times out of ten, therefore showing itself to be positively phototactic.

This difference in orientation becomes more marked if the total number of degrees, that is, the *amount* of positiveness and negativeness of emergence from the circle is used as the basis of reckoning, instead of only the number of times of emergence. Such a quantitative computation is shown in Table XII.

TABLE XII

Amount and kind of orientation to directive light exhibited by various species of planarians in 396 trials

	Number of trials	Total degrees positive	Total degrees negative	Percentage of degrees neg.	Percentage of degrees pos.
Negative worms (Dendrocoelum, Planaria, Phagocata)...	386	566	10157	94.7	5.3
Positive worms (Bdelloura)...	10	397	50	11.2	88.8

Although the actual number of trials for Bdelloura in this table is small, they are characteristic of what was observed in a large number of unrecorded instances.

The amount a planarian may deviate from the direction in which it is pointed, depends upon the direction of the light impinging upon it. A negative species deviates from a straight course least when headed away from the source of the light and most when headed toward it, while an intermediate degree of deviation occurs when the direction of the light is at right angles to the long axis of the worm. In the case of Bdelloura the converse is true, as shown in Table XIII.

Rate of Locomotion. In obtaining the rate of locomotion of worms subjected to directive light, the double aquarium apparatus

just described was used. After the worm to be tested had been placed in the inner aquarium and had begun gliding, it was so oriented that the tip of its posterior end came precisely over the center of the subjacent circle 10 cm. in diameter. The exact time of its departure from the center of the circle was then noted and the instant thereafter that the tip of the posterior end passed over the circumference of the circle was again taken and the worm's course plotted at once on a duplicate circle sheet. Each worm was given four trials in this manner, being started in four different directions, toward the light, away from the light, and with the long axis of the body at right angles to the light, first with one side to the light and then with the other.

TABLE XIII

Amount of average deviation in 2400 trials expressed in degrees of a circle, exhibited by negative planarians, (Dendrocoelum, Planaria and Phagocata), and a positive one (Bdelloura) when pointed toward, away from, and at right angles to the source of light

Direction in which the worm was pointed with regard to the light	At right angles		
		Toward	Away from
Negative planarians, degrees.....	48.1	128.7	27.3
Positive planarians, degrees.....	49.	39.3	132.1

The time of the worm's emergence from the circle was not taken with a stop-watch because the observer's hands were otherwise occupied. Instead a small clock, ticking half-seconds, was placed conveniently near. By counting the number of ticks during the interval of the worm's transit from the center to the circumference of the circle the time consumed could be determined within less than a half-second. After tracing the worm's course on a duplicate circle sheet and measuring the same by means of a map measurer, a unit of distance was obtained, which together with the known unit of time consumed in covering this distance, furnished all the data necessary for computing the rate of locomotion.

Ten representatives of *Dendrocoelum lacteum*, *Planaria maculata*, *Phagocata gracilis* and *Planaria gonocephala* respectively were given four trials apiece by the method just explained. The results are presented in Table XIV. From the 160 records thus obtained it becomes evident that the average rate of locomotion

is greatest when the worms are pointed toward the light, and least when they are pointed in the opposite direction, while an intermediate rate occurs when they are started at right angles to the light.

This result is at variance with the findings of Parker and Burnett ('00, p. 381), who incidentally reported that *Planaria gonocephala* when started away from the light traveled faster than when started toward the light.

TABLE XIV

Average rate of locomotion, expressed in mm. per sec., of various species of planarians when started forward, away from, and at right angles to the source of directive light of 27 c.m. intensity.

Species	Dendrocælum lacteum	Planaria maculata	Phagocata gracilis	Planaria gonocephala	Total average
<i>Direction in which the worm was pointed with reference to the light</i>					
At right angles	0.855	1.475	1.445	0.980	1.19
Toward	0.910	1.505	1.430	1.205	1.26
Away from	0.795	1.440	1.310	1.090	1.16

It was further found that, regardless of the direction in which the worms were started, there was a gradual decrease of the rate during the four successive trials. The order in which different worms were oriented during the four trials was arranged so as to neutralize the possible effect of the sequence in the direction started. In Table XV the data for 200 trials are arranged to express this slowing down of the rate.

TABLE XV

Average decrease in rate of locomotion for 50 planarians during four successive trials while subjected to directive light of 27 c.m.

Number of trial.....	First	Second	Third	Fourth
Average rate in mm. per sec.....	1.140	1.130	1.075	1.070

Various factors influencing the rate of locomotion, such as the intensity of light, the size and species of the worm, the amount of pigment present in the body and the general physiological state of the animal under experimentation, will be more suitably discussed in other connections.

Change in Character of Course. When several specimens of *Phagocata* were placed in a square aquarium which received light solely from one side, their first movements were plainly negative, that is, away from the light. After a brief interval, however, it was seen that apparently as many worms were going toward the light as in the opposite direction. In fact an actual count showed that in a certain interval of time 43 worms passed a central point going toward the light while 44 passed the same point in the opposite direction. This apparent change in the character of the course was probably due, not to any change in the degree of negativity of the animal, but rather to the fact that the impulse to keep moving in some direction is stronger than the impulse to negative phototaxis. Consequently when the limit of the aquarium in a negative direction is reached a worm, since it normally travels in straight lines or sweeping curves and does not turn around and around in one spot, continues its locomotion in the direction of least resistance, namely, back toward the light. It will be remembered that Loeb ('93b) has called attention to this fact by saying that planarians are not negatively "heliotropic" in a strict sense because they do not remain as far away from the source of light as they can get.

Among various observations made with other ends in view, there were numerous incidental cases of a normally negative worm making an unexpected positive response even from the first moment of being subjected to the light stimulus. This occasional positiveness is clearly apparent from the general fact already noted that four times out of a hundred the average negative planarian turns toward the light.

Two definite instances of a reversal in the character of response may be cited.

The first was the case of a *Phagocata* in the double aquarium, which became increasingly positive through twelve successive trials. Its average emergence from the circle for the first four trials was 45° , which is a normal negative result, since 90° represents complete indifference. In the next four trials, however, the average was 100° , that is, slightly positive, and in the last four, 124° , which is decidedly positive, as shown diagrammatically in Fig. 6.

In the other instance an individual worm, *Planaria gonocephala*, made the erratic average emergence from a circle of 145° , just 35° short of absolute positiveness. This worm was carefully isolated and tested again four days later under identical external conditions when it was found to have returned to a normal negative condition by showing an average record of 56° .

Accuracy of Orientation. It was found to be frequently the case that when negative worms were subjected to directive light their first movement instead of being directly away from the source of light formed a path in a diagonal direction. This tendency to

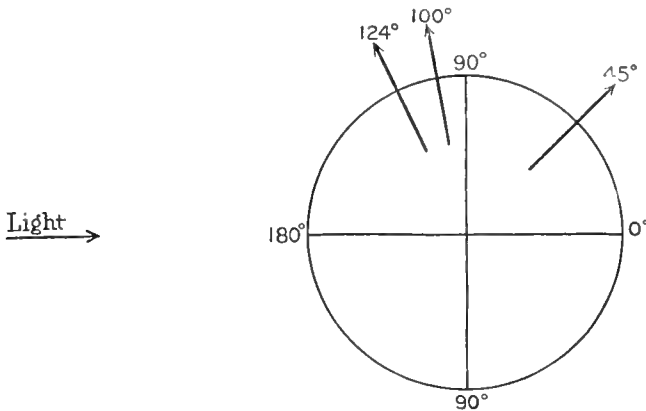


Fig. 6 The arrow at the left represents the constant direction of the light. In each of the three sets of trials each worm was headed successively toward 0° , the upper (in the diagram) 90° , 180° , and the lower 90° . The point of average emergence for the first set of trials—supposing the records of the lower semicircle to have been transferred to the upper semicircle—was at 45° , of the second set, at 100° , and of the third set, at 124° .

travel diagonally away from the light has also been noted in the case of the earthworm by Smith ('02, p. 469).

If the negative phototaxis of planarians is to be explained on the theory of tropisms, and if, moreover, the eyes, as Hesse ('97) maintains, are the principal organs which, when unequally illuminated, cause the directive response, it may be shown that possibly the arrangement of the crescentic pigment shields around the sensory cells of the eyes is such that equal stimulation of both eyes is just as certainly received by the worm when it is in a position diagonal to the light as when it is pointed directly away from the light.

By reference to Fig. 7, in which the relative size of the eyes is somewhat exaggerated and made diagrammatic for sake of clearness, it will be seen that no more light reaches the sensory cells of either eye from position *A*, the diagonal position, than from position *B*, and that it is only when the light comes from some source more lateral than *A* that the left eye receives more illumination than the right.

This view may furnish a possible explanation of the diagonal paths representing imperfect orientation among planarians, but it can in nowise apply to the case of earthworms since in them direction eyes are absent.

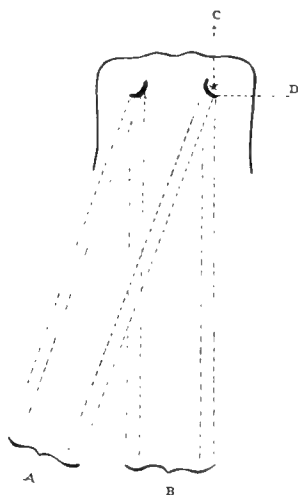


Fig. 7 *A*, diagonal direction of light; *B*, posterior direction of light; *C*, location of sensory cells; *D*, pigment shield.

Degree of Wandering. The degree of wandering decreases with an increase of intensity. It may be found approximately through the degree of error in orientation in a unit space under different intensities of light, for perfect orientation signifies the minimum of random wandering and, conversely, the greater the error of orientation the greater the probable wandering.

The error of orientation expressed in percentages was computed as follows. With a negative worm emergence from the circle at

a point directly opposite the light was reckoned as 0 per cent of error, whereas emergence at a point directly toward the light was reckoned as 100 per cent, or a maximum of error in orientation. The orientation value at these two extremes having been established, the percentage of error which occurs when the worm emerges at any intermediate position on the circumference of the circle may be easily determined.

TABLE XVI

Average degree of error in orientation made by various species of planarians during 360 trials in directive light of different intensities

	Percentage of error in orientation		
	When started toward the light	When started away from the light	Average
3.3 candle meters.....	34	11.5	22.7
27.0 candle meters.....	32	12	22.0
53.0 candle meters.....	31	10	20.5
Average.....	32	11	

From this table it appears that there is three times as much wandering, or error of orientation, by worms headed toward the light, as by those headed away from it. This doubtless indicates that orientation is a more complicated process in the former case than in the latter.

Duration of Activity. Superficial observation is sufficient to establish the fact that different species of planarians when set into activity in directive light show decided differences with regard to the length of time they normally continue in motion before coming to rest. Among the forms experimented upon, *Bdelloura* came to a stand-still in light soonest and *Phagocata* latest.¹ Fatigue in itself is by no means the inevitable result of continued activity on the part of an organism. For instance, Hodge and Aikens ('95) observed a *Vorticella* continuously for 36 hours, during which time its regular ciliary and contractile movements continued uninterruptedly, while Rádl ('01) found that the eye of *Daphnia* when

light was flashed upon it vibrated as vigorously after the experiment had been repeated 410 times in close succession as it did at first.

An attempt was made with *Planaria maculata* to see how long activity would continue in a succession of trials in directive light. The worm was started on the middle of an aquarium floor and allowed to glide in any direction. As soon as it stopped and assumed the relaxed contour of the resting worm, the time required for the journey being noted, it was immediately returned to the starting point. Subjected to this treatment, the worm made 39 trips, which in general occupied an ever decreasing length of time, ranging from 18 minutes to $1\frac{3}{4}$ minutes, or an average of 5 minutes and 53 seconds each. When returned to the starting point the fortieth time the worm refused to start. Although in this experiment, which lasted $4\frac{1}{2}$ hours, the worm became gradually less responsive to the mechanical stimulus of the brush by means of which it was transferred to the starting point, its fatigue did not materially affect the negative character of its response to light.

Time Required to Leave a Unit Circle. In obtaining the data on this point, the apparatus and method already described (p. 73) were employed. It was found that when worms of different species were subjected to three different intensities in immediate succession the degree of intensity did not prove to be as important a factor as fatigue in determining the average number of seconds necessary for the worm's exit from a circle 10 cm. in diameter.

During the series of experiments upon this point care was exercised so to vary the succession of intensities that the effect obtained could not be attributed to any cumulative increase or decrease of intensity. Thus, on one day the order of intensities was 1, 2, 3, on the next 2, 3, 1, and on the third, 3, 1, 2. In Table XVII the data obtained are arranged on the left with reference to the actual intensities employed and on the right with reference to the succession of trials made upon the various species which are designated in the middle column. The averages in the table are each made up of four records.

It will be noted that *Phagocata gracilis* and *Planaria gonocéphala* are, according to these figures, less subject to fatigue than *Dendrocœlum lacteum* or *Planaria maculata*.

Manner of Coming to Rest. Loeb ('93b) and others have shown that planarians under the influence of directive light generally come to rest in regions of lessened intensity. A few experiments were made bearing on this point. By means of screens and backgrounds, both black and white, a rectangular glass aquarium was arranged so that the area of least intensity was plainly localized and could be varied in different ways. In Fig. 8 are shown (1) the places where worms (*P. gonocephala*) which had been started together in the middle of the dish finally came to rest; (2) the number of worms in each locality; and (3) the different combinations of backgrounds and screens used in each of the experiments.

TABLE XVII

Relative effect of fatigue (at right of table) and change in intensity of light (at left of table) as shown by the average number of seconds required for individuals of various species of planarians to leave a circle 10 cm. in diameter

INTENSITY			SPECIES	GROUPS OF TRIALS		
3.3 c.m.	27.0 c.m.	53.0 c.m.		First	Second	Third
<i>seconds</i>	<i>seconds</i>	<i>seconds</i>		<i>seconds</i>	<i>seconds</i>	<i>seconds</i>
63	65	64	<i>Dendrocoelum lacteum</i>	54.5	63	72.5
40	43	41	<i>Planaria maculata</i>	37	42	45.5
40	38	46	<i>Phagocata gracilis</i>	38	44	42
52.5	47	49	<i>Planaria gonocephala</i>	46	52.5	50
65	64	67	Average	58.5	67	70

Wherever shaded borders are indicated the aquarium was surrounded on five sides by black screens and likewise on the sixth side except for a narrow space admitting the light, the direction of which is indicated by arrows; in a similar fashion, where unshaded borders appear, light-reflecting screens enclosed five sides.

It will be seen at a glance that the great majority of the worms placed in directive light come to rest as far from the light as possible. That this is due to the directive power of light is at once apparent by comparing *A*, *B* and *C* with *D*, where the light was non-directive. The darkened area was selected whenever the directive force of the light did not prevent, as in *A*, *C* and *D*.

photic stimuli, due to the worm's lowered physiological state and a chance turn toward the light. In fact the final position taken by 49 fatigued worms with reference to the source of light, showed that only five of them, or 10+ per cent, pointed away from the light while 15 (30+ per cent) were headed toward the light and 29 (59+ per cent) stopped indifferently at right angles to it. It is quite probable that among the external factors that influence a worm to come to a halt, light plays an exceedingly insignificant rôle, as compared with the stimulus of contact or some stimulus, probably chemical, given out by other worms in close proximity.

One curious instance was observed, however, in which light was apparently of more importance than contact or other stimuli in determining the place of coming to rest. A large crystallizing dish half full of water was left over night with a few planarians in

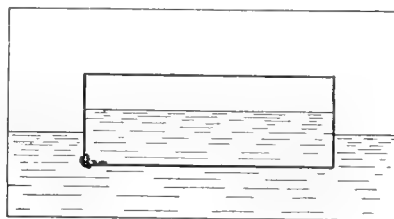


Fig. 9

it. Floating on the surface of the water in this dish was a small Petri dish, in which a few more planarians were isolated. In the morning the worms in both vessels were found grouped at the same region on the outside and inside of the smaller dish, as shown in Fig. 9.

This curious distribution on both surfaces of the Petri dish could not be due to chemical stimulus exerted by one group of worms on the other, and there seems to be no particular reason why a thigmotactic reaction should have caused them to assemble in such a way. The locality chanced to be one, however, where the intensity of the light was considerably reduced; this seems to offer a reasonable explanation of the observation.

Bdelloura in coming to rest shows an entirely different behavior. When left over night free to wander in an aquarium half of which

had been previously covered with black cloth to exclude most of the light, this species was found in the morning in the light area, a behavior exactly the reverse of that shown by fresh-water planarians. Another peculiarity of this species is that individuals in coming to rest arrange themselves in compact rosettes with the anterior end of the body pointed toward the circumference of the rosette, while the sucker-like posterior end remains attached near the center of the group. They are so delicately responsive to mechanical stimuli that any slight disturbance of one member of such a rosette is sufficient to throw the whole group into activity. The advantage to the individual worm of such a habit of arrangement in coming to rest, is evident.

Finally, *Bdelloura* was repeatedly seen on taking the resting position to point directly toward the light with the anterior end of the body raised and the posterior end flattened out into a sucker-like expansion.

Summary. Fresh-water planarians (*Dendrocœlum*, *Planaria* and *Phagocata*) are negatively phototactic while *Bdelloura* is positively phototactic.

Negative planarians deviate most from the direction in which they are started if pointed toward the light and least if pointed away from the light, an intermediate deviation occurring when they are pointed at right angles to the light.

The rate of locomotion is greater when worms are headed toward the light than when they are headed away from it.

During successive trials the rate of locomotion decreases.

Negative planarians frequently take an apparently positive course because the impulse to move in any direction is greater than the phototactic impulse.

The normal negative phototaxis of a worm may change temporarily to positive by reason of some physiological state which is not obviously referable to external stimuli.

The greater the intensity of the light the less worms wander in their course. When they are headed away from the source of light, there is less error in the precision of their orientation than when they are started toward it.

Planarians frequently travel away from the source of light diagonally instead of directly.

Bdelloura continues activity in the light for a much shorter time than *Phagocata*.

When subjected to successive trials the period of a planarian's activity decreases.

Change in the intensity of light is less important than the effects of fatigue in determining the time required for a worm to leave a unit circle. When fatigued, worms often become indifferent to light, coming to rest less frequently in an oriented position with reference to the light than in an unoriented one.

Fresh-water planarians come to rest as far away from the source of light as possible and, if the directive stimulus does not prevent, in the region of least illumination.

Bdelloura candida, on the contrary, comes to rest in regions of greater rather than of less illumination; usually worms of this species arrange themselves in compact rosettes with the anterior ends pointed outward.

B In Changing Directive Light

The light acting upon planarians in their natural habitat must necessarily be a variable factor of great complexity, since its intensity changes constantly throughout the day, while the position of the sun relative to various surfaces which reflect light is also continually shifting.

The fact that planarians, to a great extent, keep out of the light, does not diminish the force of this statement, for whatever the part played by light in their behavior, it must always be an exceedingly varied and complex one.

Changes in the Intensity. When a worm is gliding away from a source of light it shows a more marked response to change of intensity when the change is made *suddenly* than when it is made gradually. In fact, it is possible by exercising patience and care to change the intensity of directive light to a considerable degree so gradually as to produce no corresponding response on the part of the worm, whereas a comparatively slight change, if abruptly effected, immediately results in the animal's performing some one or more of the acts in its repertory of behavior, such as halting, wigwagging, etc.

In all the experiments made upon the effects of change of intensity in directive light, more responses were found to occur when the intensity was decreased than when it was increased. This is in agreement with the experiments already described relating to the critical region between two intensities, in which it was found that worms show a greater number of responses when going from a higher into a lower intensity than vice versa.

Bdelloura is particularly sensitive to changes in intensity. It is necessary to throw a shadow on a moving worm only momentarily to cause it to perform vigorous wigwag movements or to change the direction of its course.

Whitman ('99), writing of *Clepsine*, suggests that the extreme agitation of this animal when a shadow is thrown upon it may be the result of natural selection, since any sudden shadow cast upon it in its natural environment may be caused by a turtle swimming overhead, to which the leech, if it is quick enough, may become attached. It may be that *Bdelloura*, which is also an ecto-parasite, has developed this extreme responsiveness to sudden decrease of intensity in a similar way.

Changes in Direction. The precision with which all the planarians in a dish may be made to pass back and forth by shifting a directive light from one side to another is a striking phenomenon, which is sure to impress anyone who sees it. By careful manipulation of the light, it is possible even to make an individual planarian follow a predetermined path in the most undeviating manner. For example, when two lights, placed near the ends of an aquarium, are alternately turned on and off, the worm will zigzag across the field, at right angles to the direction of the lights, while under a moving light it may be made to turn around and around, almost as if its posterior end were a pivot, to trace figure 8's and curves of various patterns, or to turn abruptly at right angles an imaginary corner.

Unlike the changes in intensity previously described the degree of abruptness in any change in the direction of the light made no apparent difference in the quality of the reaction, since any change in direction, however gradual, met with an immediate response on the part of the worm. Indeed it was necessary to abandon an

attempt to illuminate one side of the worm alone because the animal invariably turned faster than it was possible to regulate the light.

The quickness with which this delicate response to any change in the direction of the light occurred was found to increase upon successive trials. A square aquarium was arranged so that it could be illuminated instantly at either end, in a room otherwise dark. With one light on, a planarian was allowed to move until it had assumed the characteristic negative direction, whereupon the source of illumination was instantly changed 180° by turning this light off and the one at the other end of the aquarium on. The time required for the worm to become headed about was noted and then a reversal of lights repeated and the interval necessary for readjustment again recorded. In a typical experiment of this kind the number of seconds required by the worm, *Planaria maculata*, to accomplish re-orientation were for 16 successive orientations as follows: 260, 70, 100, 60, 65, 110, 60, 85, 70, 105, 80, 60, 50, 40, 45, 35. The sum of the first eight is 810 sec., that of the last eight, only 485 sec.

Summary. Planarians show a greater response to sudden change of intensity than to gradual change. This response is more pronounced when the intensity is lowered than when it is raised.

Bdelloura is particularly affected by sudden changes of intensity.

Planarians respond with great precision to changes in the direction of the light, and as promptly when the change is gradual as when it is abrupt.

The period required for re-orientation to changes in the direction of light, diminishes upon repetition.

C In Combination with Other Responses

It is impossible to subject planarians to the influence of light alone. The best that can be done is to render extraneous factors as uniform as possible. For example, so long as a moving worm is kept upon a horizontal surface there can be no directive geotactic stimulation, because the worm is moving in a plane at right angles

to the force of gravity. The moment the worm begins to glide up the sides of an aquarium, however, the relation of the axes of its body to the center of the earth changes and directive geotaxis results.

No systematic attempt was made to analyze compound stimuli, for such a study would overstep the boundaries set for the present inquiry. Nevertheless certain facts bearing on this point were incidentally noted and these may properly be detailed here.

Geotaxis. In a majority of cases, *Planaria gonocephala* seems, after several hours of exposure to the dark, to be positively geotactic, and after several hours of exposure to light, negatively geotactic, as shown in the following series of observations.

A cylindrical aquarium jar 20 cm. in diameter and 40 cm. high was placed before a moderately lighted window and stocked with a freshly obtained supply of about 300 worms. No stones, sand, or water-weeds, which would afford places of concealment, were introduced. At intervals during the next 10 days the distribution of the worms was recorded and these records are brought together in Table XVIII.

TABLE XVIII

The distribution of about 300 planarians (Planaria gonocephala) in an aquarium, as observed forenoons and afternoons during 10 days. The figures express percentages

PLACE IN THE AQUARIUM Time of day	TOP		SIDES		BOTTOM	
	a. m.	p. m.	a. m.	p. m.	a. m.	p. m.
April 26.....		51		11		38
April 27.....	61		13		26	
April 28.....	74	3	11	15	15	82
May 1.....	72	29	6	7	22	64
May 2.....	63	39	12	20	25	41
May 3.....	43		16		41	
May 4.....	50		16		34	
May 5.....		31		13		56
Average.....	60.5	30.6	12.3	13.2	27.2	56.2

The forenoon census was taken about 8 o'clock, when the worms were re-arranging themselves after the darkness of the night, while the afternoon records were made about 4 o'clock, when the worms

had been all day in the light. The average at the bottom of the table indicates, first, that an approximately equal percentage of worms was found on the sides of the aquarium at both times of day, which may therefore be left out of the reckoning, and, secondly, the occurrence of a significant migration during the interval between 8 a. m. and 4 p. m., demonstrated by the distribution of the worms at the top and bottom of the jar respectively. According to the data obtained, at least 30 per cent of the worms in the top group must have become positively geotactic and gone to the bottom during the day.

A later set of experiments in which an aquarium was kept swathed in black cloth during the day showed less migration. The conclusion naturally follows that geotaxis is more likely to occur in the presence of light than in its absence. Whether there is a regular diurnal vertical migration among planarians in nature, as Birge ('97) and Schouteden ('02) found to be true for freshwater entomostraca, and various authors⁴ for different forms in marine plankton, remains unknown. It is probable, however, that planarians ordinarily remain quiescent on the under sides of stones or in other shaded places for considerable intervals of time, coming under the influence of light only when started into activity through some other stimulus.

A worm placed in an aquarium with square sides and left free to travel undisturbed on the bottom or the sides occupies the sides more frequently than the bottom.

In a trial to test this point, an aquarium was used, the bottom area of which measured approximately five times that of the sides. The course pursued in this aquarium by one worm (*P. gonocephala*) in directive light and covering 1340 cm., was plotted and the percentage of distance traveled on the sides was found to be practically equal to that traveled on the bottom, notwithstanding the fact that the animal was started in the middle of the bottom, where it had five times as much available territory to travel over as on the sides. Other things being equal, therefore, this worm showed itself five times as ready to travel on the sides of the aquarium as on the bottom.

⁴Groom and Loeb ('90), Loeb ('93a), and Parker ('02).

The existence of such a decided geotactic tendency should not be forgotten when trying to determine the part light plays in planarian behavior.

Again, it was found that there was less accuracy of orientation to directive light while the planarians were on the sides of the aquarium in a position parallel to the light rays than while they were on the bottom.

Their behavior in the former case was the resultant of at least two known stimuli, gravity and light, whereas gravity was practically eliminated when they glided on the floor of the aquarium. In the experiment cited under the preceding paragraph 92 per cent of the distance traversed by the worm on the bottom of the aquarium was in a direction in general away from the light, as contrasted with only 79 per cent when it was traveling on the sides of the aquarium. This difference of 13 per cent may represent roughly the necessary correction for geotaxis, in order to ascertain the influence of light alone.

Thigmotaxis. Contact with the substratum is an almost constant condition of planarian activity. Occasionally worms may be seen dangling free at the end of a mucus-thread, as commonly occurs among many fresh-water snails; sometimes they may fall helplessly from the surface-film to the bottom, but definite contact with something firm is the rule during their ordinary locomotion.

A change in the degree of this contact, and consequently a production of thigmotactic stimulation, may come about in two ways: the surface on which the animal glides may present irregularities, such as increased roughness or a different degree of solidity, or the worm itself may vary in the extent of body-surface which it brings into contact with the substratum. This latter method of causing thigmotactic stimulation applies especially to *Bdelloura*, which has the habit of frequently alternating a leech-like looping movement with ordinary gliding, thus changing its contact relations and probably producing a thigmotactic stimulus in consequence.

As already mentioned, *Bdelloura*, when subjected to sudden dark, usually detaches itself from its support and wriggles vio-

lently in the water. It is uncertain how far this behavior is attributable to light alone or to some combination of light and thigmotaxis.

This phenomenon of compound stimulation occurs in a less pronounced way whenever a change of light intensity results in the "wigwagging" response common to planarians. The same uncertainty prevails as to how far the subsequent behavior of the worm may be due to the direct stimulation of light and how far to thigmotactic stimulation primarily and to light stimulation secondarily. It is evident, then, that under any circumstances there is such a close interrelation of stimuli that an accurate analysis of the consequent behavior is difficult.

Further evidence of the close relation between different kinds of stimuli is afforded by the fact that planarians are more responsive to the mechanical stimulus of a slight jar when the entire ventral surface of the body is in contact with the substratum than when the anterior end is lifted up and waving about. Apparently the greater the degree of contact the greater is the effect of a jarring mechanical stimulus.

This point was demonstrated by means of a small aquarium mounted on a turntable, such as is used in "ringing" microscopic slides, in such a way that it could be rotated with great ease and delicacy. A light from one direction only was projected upon the single planarian placed in the aquarium. Any attempt to change the angle of light by rotating the aquarium ever so slightly resulted instantaneously in a momentary halt on the part of the worm, provided it happened to be gliding with its ventral surface entirely in contact with the floor of the dish. If, however, the rotation was made when the anterior end of the worm was lifted, the halt did not so readily occur. This response was of such delicacy that with a little practice it was possible to halt the anterior end of a worm without disturbing the continuous progress of the posterior end! That this halting was due to thigmotaxis rather than to any rheotaxis induced by the movement of the animal against the relatively stationary water particles, is shown by the fact that the reaction was more pronounced when the anterior end of the body was held flat than when it was raised and so brought more under the possible influence of a water current.

Finally, it may be recalled that in a preceding section data were given (Table X, p. 68) to show that there is more response to light while worms are upside down on the surface-film than when they are in contact with the bottom of the aquarium, a difference probably referable in large measure to the different thigmotactic relations in the two cases.

Goniotaxis. Goniotaxis is a term introduced by Pearl ('03, p. 561) to define a particular kind of thigmotactic response in which the "different parts of the body are brought into such positions that they form unusual angles with each other," as when a planarian occupies the angle formed between a side and the bottom of an aquarium.

There is no doubt that the peculiar movements resulting from the goniotactic stimulus directly modify the phototaxis of the worm. Once in the angle of an aquarium a planarian becomes increasingly indifferent to light. In one series of records, showing how a considerable number of planarians came to rest, it was found that the majority came to rest in an "angle" and that out of this number 78 per cent failed to orient to the light. The stimulus of the "angle" was greater apparently than the stimulus of light.

Furthermore, it is to be noticed that goniotaxis is always more effective if the worm is in a lowered rather than in a heightened physiological state, for whenever a planarian is freshly introduced into an aquarium and is in an aroused condition on account of the mechanical stimulation necessarily given it in transference, it will pass over angles and crevices with total indifference, all the while responding plainly to light. As soon as it has become fatigued, however, if its path chances to cross an angle or crevice it exhibits goniotaxis at once by slowing down and remaining in the new situation, as if caught in a trap, with complete disregard of the continued action of directive light.

Chemotaxis. Pearl has made an extensive study of this phase of planarian behavior and suggests that the well-known planarian habit of collecting in groups may be explained on the supposition that a resting planarian is surrounded by a halo of chemical ema-

nations which serve as a direct stimulus to other planarians, attracting them and causing them to come to rest in groups.

In this connection it is worth mentioning that several times when *Dendrocoelum lacteum* was put in an aquarium with other species of planarians, the individuals of this species would later be found gathered into a separate group by themselves. This manner of isolation was also repeatedly noticed in examining on the under sides of stones taken from the pond at Falmouth, Mass. A similar segregation of species in the case of *P. alpina* and *P. gonocephala*, was noted by Collin ('91). He says ('91, p. 180) "Iijima fand diese beiden Arten zusammenlebend, während sie im Harz stets getrennt vorkamen; auch in der Gefangenschaft schien die *P. alpina* die grössere *P. gonocephala* in demselben Behälter zu meiden und ihr ängstlich auszuweichen." It would be difficult to explain how these planarians avoid each other so as to fraternize in this fashion, except on the basis of some delicate chemotactic response which caused them to halt when they entered the chemical halo of their own kind, but not to do so in the different chemical halos of other species. As in the case of goniotaxis, the manifestations of phototaxis may be entirely superseded by the effect of feeding (Chemotaxis). When once a hungry planarian, driven by directive light into the neighborhood of a crushed snail, becomes subjected to the chemical stimulus arising from the fluids of that object as they are disseminated through the water, it seems to become suddenly indifferent to the light, owing to the greater influence of the chemical stimuli.

The same inhibition of the influence of light by a chemotropic response to food has been observed by Parker ('03) on the mourning-cloak butterfly, *Vanessa antiopa* L. He says ('03, p. 457) "when a butterfly alights on a bough, it orients in the sunlight with the usual precision. Should the sap be running from a near stem, the insect is very soon attracted to the spot, begins feeding, and moves about from that time on with no reference to the direction of the sun's rays. Thus, when feeding or near food the butterflies do not respond phototropically." Furthermore Darwin ('81, p. 23) observed that earthworms are less disturbed by light while feeding or during copulation than at other times.

The foregoing examples illustrate only a few of the many modifications of light responses due to the interference of some other stimulus.

Summary. In judging the effect of any stimulus upon an animal it is necessary to have constantly in mind the accelerating or inhibiting effects of other stimuli which may be influencing the organism at the same time. In the case of planarians some of the responses known to be intimately connected with phototaxis are geotaxis, thigmotaxis, goniotaxis and chemotaxis.

Planaria gonocephala shows itself to a certain extent negatively geotactic after several hours of dark and positively geotactic after a similar interval of light.

When given horizontal and vertical surfaces of equal extent, worms travel more on the vertical surfaces.

Their accuracy in orienting themselves to light while subjected to geotactic stimulus on a vertical surface is less than when they are traveling on a horizontal surface, where the directive geotactic stimulus is eliminated.

Thigmotactic stimulus may result either from an environmental change in the substratum, or a change in contact caused by the worm itself whereby its relation with the substratum is varied.

There is a close interdependence of the various stimuli which may be acting on an animal at the same time.

Behavior may be the direct consequence of light or the indirect result of light combined with the direct effect of a thigmotactic stimulus indirectly brought about by some change in the intensity of the light.

The greater the degree of contact with the substratum the more responsive a planarian becomes to the mechanical stimulus of jarring, but the less to the stimulus of light, as shown by comparing the behavior of worms on the surface film with their behavior on the aquarium floor.

Goniotaxis has an inhibitive effect on phototaxis; this effect becomes more apparent as the worm reaches a condition of fatigue, phototaxis meanwhile becoming less apparent.

Dendrocœlum lacteum exhibits a remarkably delicate response (Chemotaxis?) in frequently coming to rest in the neighborhood of its own kind.

Hungry planarians in the presence of food have their phototaxis entirely obscured.

3 KINDS OF BEHAVIOR

In the two preceding sections, treating of Photokinesis and Phototaxis, respectively, animal behavior, as illustrated by the effect of light upon planarians, has been taken up from the point of view of the stimulus. In the two following sections, on the other hand, the reactions of planarians will be dealt with from the standpoint of the animal rather than from that of the stimulus.

To this end a classification of the behavior of planarians in light is here presented based upon (A) generic and specific differences, and (B) individual differences.

That there are morphological differences which fall naturally within the lines of this classification has long been recognized, indeed, the criteria used in classification by systematists are based almost exclusively upon such differences, while relatively little importance has been attached to differences in the behavior of animals.

As already mentioned in the historical review, Loeb ('94), in dealing with the differences of behavior which characterize the two genera, *Planaria* and *Thysanozoön*, pointed out that decided physiological variation may appear in forms closely related morphologically. The same fact had been previously emphasized for the case of the pulmonates by Willem ('91). Obviously such physiological variations do not furnish reliable criteria for the systematist, since they are so largely dependent upon environmental causes, and furthermore the work of the systematist is usually done upon dead animals. Nevertheless some interesting relations between behavior and systematic position await the student who approaches the study of animal behavior from this direction.

Strictly speaking, all behavior is individual behavior. In this sense it is manifestly incorrect to speak of the behavior of a genus or of a species *per se*.

The behavior of individuals may, nevertheless, be classified into responses which are characteristic of all the members of a genus,

or again into responses which are characteristic of only one species of a genus and not necessarily of other species of the same genus, and, finally, into those peculiar to the individual as such, which may not in all particulars be shared by other representatives of the species to which the individual in question belongs. It is in this sense of the terms generic, specific and individual, that behavior will be taken up in the present section.

A Generic and Specific Behavior

In the present inquiry a basis for generic comparisons is afforded by a study of the behavior of individuals of four different genera, namely, *Planaria*, *Dendrocœlum*, *Phagocata* and *Bdelloura*, while some idea of specific differences is made possible by comparing the behavior of individuals of the two species *Planaria maculata* and *Planaria gonocephala*. In the cases of *Dendrocœlum*, *Phagocata* and *Bdelloura* it is obvious that the conclusions drawn are based in each instance upon the behavior of representatives of a single species under each genus. The question may be properly raised as to how far such conclusions indicate generic behavior and how far specific behavior. Conceding that from the data obtained exact deductions may not be drawn, the fact still remains that the three species, *Dendrocœlum lacteum*, *Phagocata gracilis*, and *Bdelloura candida*, are separated from each other by generic gaps, such that the differences exhibited by these species may be regarded as generic in degree. The point unestablished, then, is whether other species of the genera in question if examined might not show that the behavior, which in these single representative species seems generic in nature, is not characteristic of other species of the same genus as well.

It will be convenient to present the data of both generic and specific behavior at the same time.

Percentage of Negativeness. The manner of obtaining this criterion of behavior has been explained in the section on Phototaxis (p. 72). It will be remembered, too, that in Table XII a comparison was made between positive and negative worms, showing the degree of their orientation to directive light. The

data there used are rearranged for the present purpose in Table XIX.

TABLE XIX

Percentage of generic and specific negativeness in worms started at right angles to incident light, as determined at the circumference of a circle 10 cm. in diam. by the average amount of their deviation from the directions in which they were started

	GENERIC DIFFERENCES				SPECIFIC DIFFERENCES	
	Dendro- cœlum	Phago- cata	Planaria	Bdelloura	Planaria maculata	Planaria gono- cephala
Number of observations.....	78	80	158	10	78	80
Total number of degrees positive...	155	238	165	397	5	160
Total number of degrees negative..	2112	1964	4070	50	2102	1968
Percentage of negativeness.....	93.1	89.6	96.1	11	99.9	84.6

Comparing the figures given in this table, a greater range of difference is seen to obtain between the two species of Planaria (*P. maculata* and *P. gonocephala*) than between the genus Planaria and either of the other negative genera, namely, *Dendrocœlum* and *Phagocata*. Although not indicated in this table, similar results appear when the *number of times* the worms went in a negative direction is used as a basis of comparison, instead of the total number of degrees of negative deviation.

Character of the Course in Directive Light. When worms were placed on the middle of a rectangular aquarium floor and subjected to a directive light their movements showed both generic and specific differences. By experimenting with one worm at a time it was possible to plot on a sheet of paper with sufficient accuracy for general comparison the entire course of the worm during a considerable period. This was done many times and typical records of such observations are given in Figs. 10-14. In such instances the worm was exposed to a light of approximately 147 c.m., placed so as to correspond to the right side of the figures. The central rectangular area bounded by the broken lines indicates the limits of the floor of the aquarium, while the smaller exterior adjacent areas represent its vertical sides so rotated as to

bring them into the plane of the floor. The course taken by a planarian is indicated by the tortuous line. The full line shows the course taken on the solid surface of the aquarium; the dot-

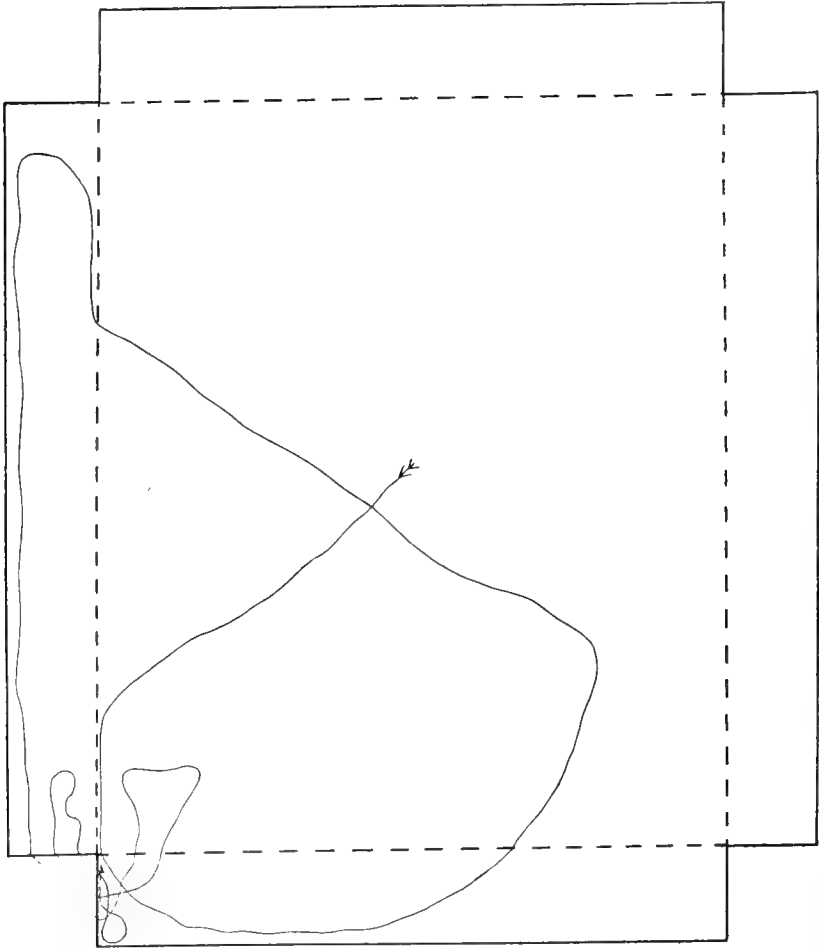


Fig. 10 *Dendrocoelum lacteum*. The shortness of the path shows comparatively little persistence in locomotion, and the direction, considerable indifference to the source of light.

and-dash lines, the course of the worm on the surface film. The dotted line indicates a hiatus in the path, made necessary by the attempt to represent on a flat surface a continuous line which traverses vertical as well as horizontal surfaces. A succession of

abrupt kinks in the line signifies that at that point the worm executed decided wigwag movements with its anterior end. The figures are reduced in size from the original records.

In Fig. 10 is given a specimen record of a *Dendrocœlum*, which

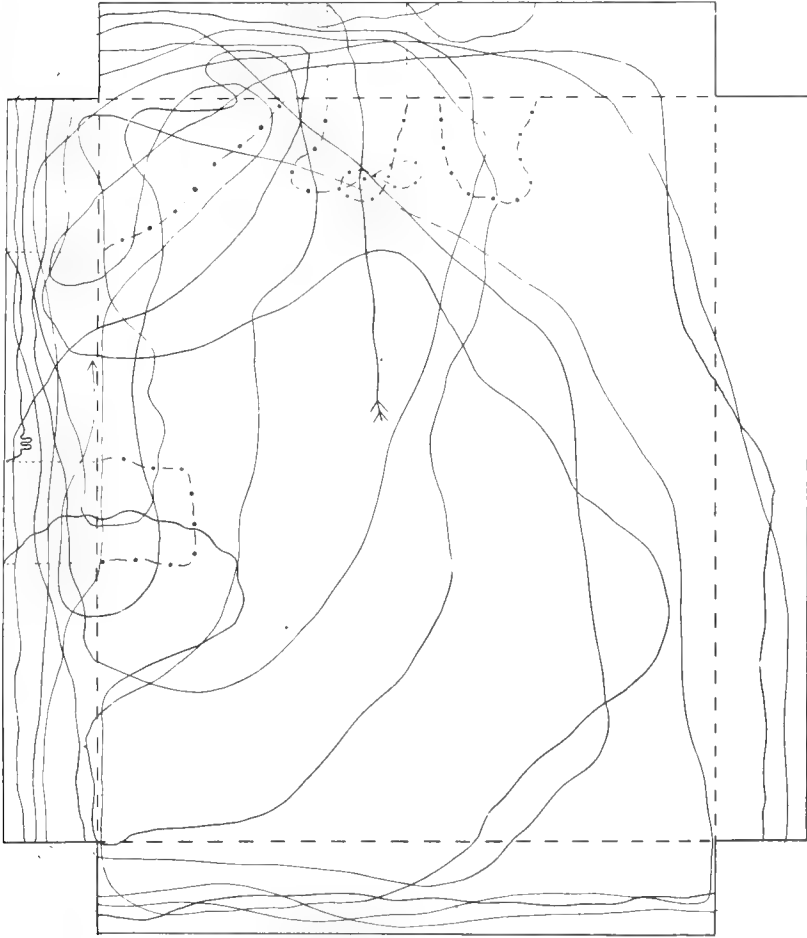


Fig. 11 *Phagocata gracilis*. This path shows great activity on the part of the worm, and, although it is mostly laid down away from the source of the light, it shows that the worm experienced no great difficulty in moving toward the light.

came to a standstill after 18 minutes of locomotion. The first movement of this worm was diagonally away from the light, but it soon came back toward the light traversing almost the entire

width of the aquarium and in doing so showed considerable indifference to the directive influence of the light. Its susceptibility to goniotactic stimulus is plainly shown by its behavior upon reach-

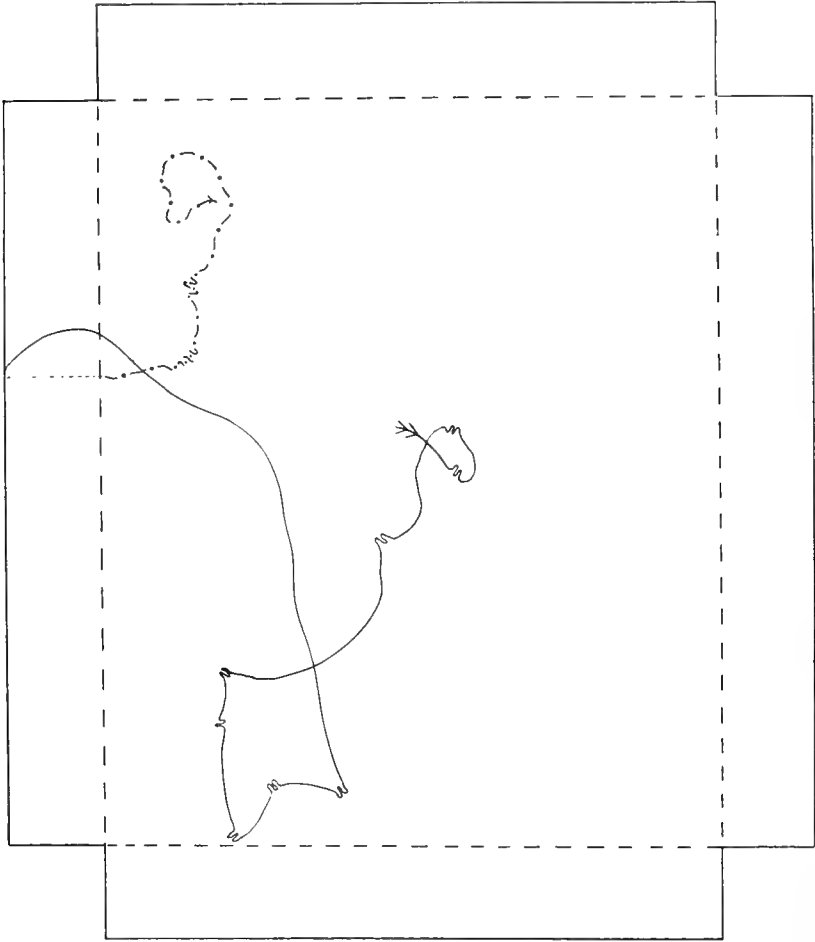


Fig. 12 *Bdeloura candida*. This path was traversed with much "wigwagging;" there was indifference to the source of light and locomotion was not of long duration.

ing the angle formed at the junction of the sides and floor of the aquarium, as well as by its manner of finally coming to rest.

A typical *Phagocata* (Fig. 11), on the other hand, exhibited

almost no goniotaxis, although the worm repeatedly crossed the line of the angle. The response to the directive influence of the light, too, was in this case even less than that of the

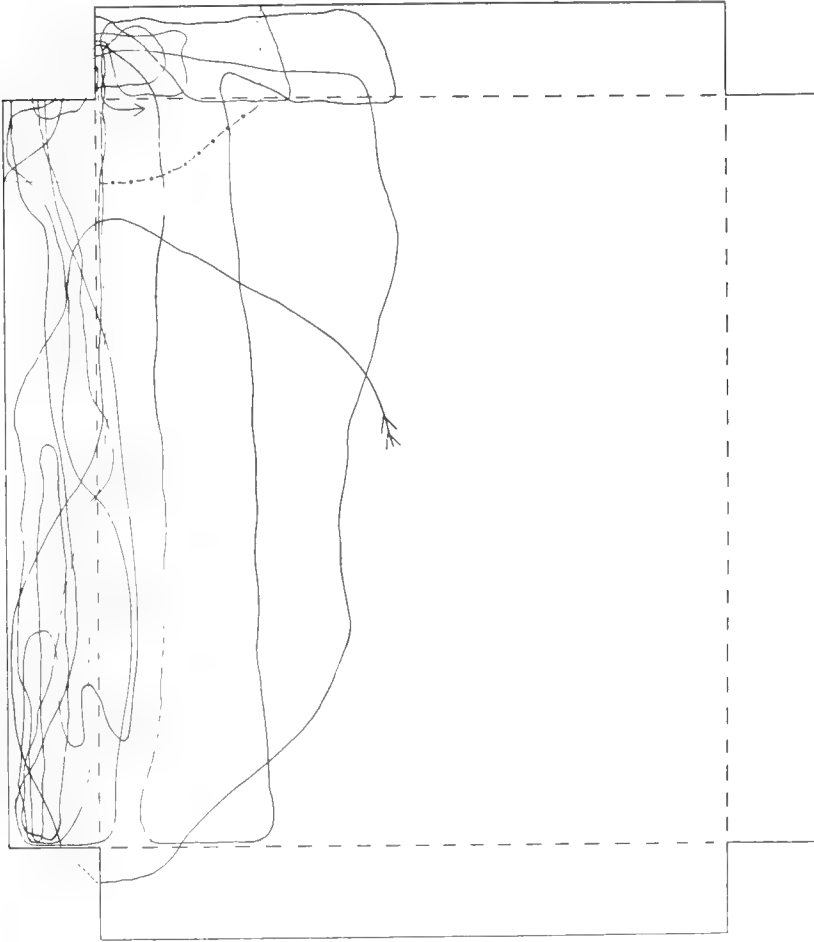


Fig. 13 *Planaria maculata*. Considerable activity was shown over this course and a decided inability to approach the source of the light beyond about the middle of the aquarium.

Dendrocœlum just described, as is evident from the general wandering character of the course. Although the Phagocata in question frequented both sides of the aquarium—that which was toward

the light, as well as the opposite side—its wanderings were in the main on the side away from the light. An hour's activity is chronicled in the record, at the close of which the worm was apparently as energetic as ever.

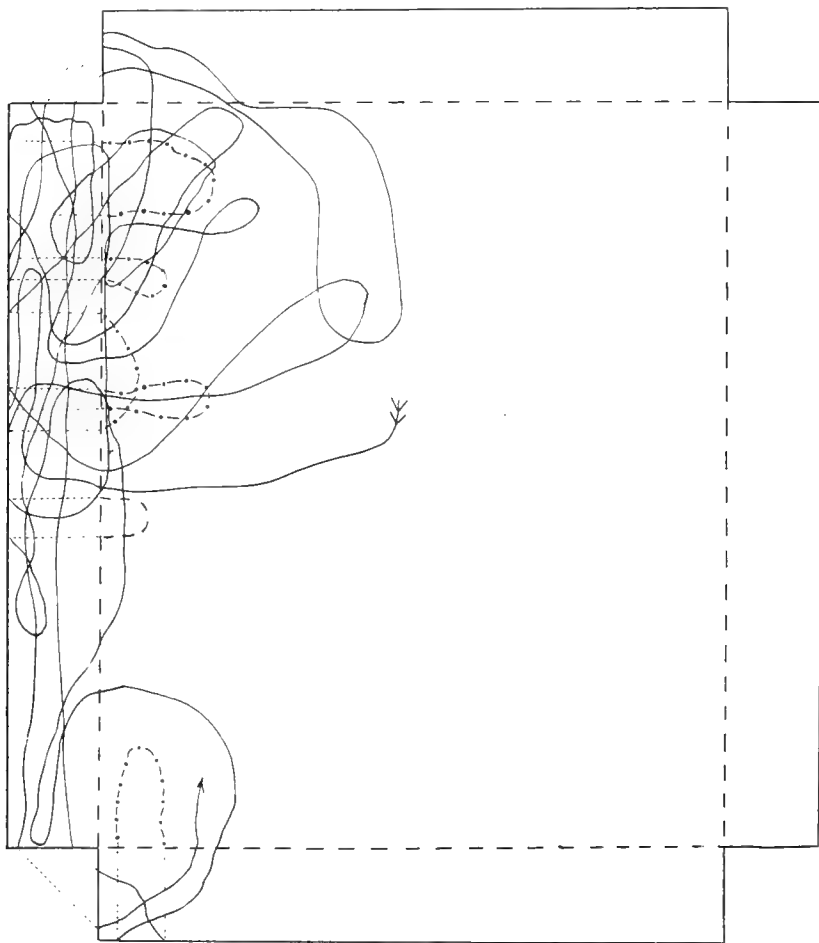


Fig. 14 *Planaria gonocephala*. The path is of the same generic type as with *Planaria maculata* (Fig. 13), and is easily distinguishable from those of *Dendrocælum*, *Phagocata* and *Bdelloura*.

Fig. 12 gives a characteristic record of the way in which *Bdelloura* behaves. The first movement of this specimen was more

toward than away from the source of the light, but very soon wig-wagging motions set in, and after every exercise of these movements, which were apparently in the nature of explorations, a *change in the direction of the course was effected*. As might be expected, such abrupt changes in direction were more difficult of execution when the worm was on the surface-film.

Characteristic movements of individuals of the genus *Planaria* are shown in Figs. 13 and 14. From these two typical records it would be difficult to select any diagnostic points which would distinguish the behavior of *P. gonocephala* from that of *P. maculata*. There is no doubt, however, that taken together the behavior of representatives of these two species presents a distinct (generic) difference from that of the representatives of the other genera studied. The most striking feature of the *Planaria* records (Figs. 13 and 14) is the high degree of response exhibited by members of this genus to the directive action of light. Although many attempts were made by the individual worms to penetrate the half of the aquarium nearer the light, yet they seemed as unable to keep to that direction as they would have been had a solid barrier been interposed between them and the light. This characteristic responsiveness to directive light helps to explain why (as shown in Fig. 8, B, p. 84) *P. gonocephala* was unable to come to rest in the area of lessened illumination as it would naturally have been expected to do.

From the cases cited in this section, at least, it may be affirmed that the generic differences are so pronounced that one could take a miscellaneous, unidentified assortment of such records and correctly assign the great majority of them to the proper genera.

Duration of Movement. When worms of different genera are subjected to the same light intensity there is considerable variation in the time required to bring them to a standstill. *Bdelloura* is usually the first to stop, followed in order by *Dendrocœlum*, *Planaria* and *Phagocata*. Of the two species of *Planaria*, *P. gonocephala*, although averaging somewhat smaller in size, usually keeps in motion for a longer time than *P. maculata*. The individual records of the duration of movement given in Figs. 10-14 may be taken as a typical set of records. They were as follows:

Fig. 10. Dendrocœlum, 18 min.

Fig. 11. Phagocata, 60 min. (still moving).

Fig. 12. Bdelloura, 15 min.

Fig. 13. Planaria maculata, 47 min.

Fig. 14. Planaria gonocephala, 60 min. (still moving).

Woodworth ('97) in contrasting the activity of *Planaria maculata*, *P. gonocephala* and *P. dorotocephala* states that individuals of the latter species remain in motion longer than individuals of the other two—an observation confirmed by Pearl ('03).

Degree of Wandering. If a worm is started at the center of a circle parallel to the direction of the light and pointing *away* from its source, then the more devious its course the more it may be said to wander. Both generic and specific differences were obtained bearing upon this phase of behavior. Selected instances of such differences are given in Table XX, expressed in average degrees of deviation upon emergence from a circle 10 cm. in diameter.

TABLE XX

The average generic and specific differences between individuals of four genera and two species of planarians expressed in degrees of deviation upon leaving a circle 10 cm. in diam. In every instance the worm was started away from the source of the light

	GENERIC DIFFERENCES				SPECIFIC DIFFERENCES	
	Dendro- cœlum	Phago- cata	Planaria	Bdelloura	Planaria maculata	Planaria gono- cephala
Average degree of deviation.....	9.4	30.0	24.6	132.0	21.6	27.7
Number of observations.....	56	46	92	10	46	46

The remarkably large deviation shown by *Bdelloura* is due to the fact that it is a positive worm. When pointed toward the light its deviation was only 39.3° , a number which would perhaps be more justly comparable with the other records in this table. But even so, it will be seen that *Bdelloura*, of all the forms observed, is the least oriented by directive light. Specific differences in the degree of wandering are in general less marked than the generic differences, according to the records in Table XX.

Rate of Locomotion. As regards rate of locomotion the records of specific differences exhibit a wide range, although not as great as that of the generic differences existing between *Dendrocoelum* and *Phagocata*.

TABLE XXI

The average rate of locomotion expressed in millimeters per second

	GENERIC DIFFERENCES			SPECIFIC DIFFERENCES	
	Dendrocoelum	Phagocata	Planaria	Planaria maculata	Planaria gonocephala
Rate in mm. per sec.....	0.850	1.395	1.272	1.470	1.075
Number of observations.....	40	40	80	40	40

It is interesting to note in this connection that Parker and Burnett ('00, p. 385) give the average rate for *Planaria gonocephala* as 1.08 mm. per sec., while Pearl ('03, p. 546) records for *P. maculata* 1.48 mm. per sec. in the case of a worm 11 mm. in length and 1.23 mm. per sec. for one 6 mm. in length. An average of these two records, that is, 1.355 mm. per sec., might perhaps be comparable with the average (1.272) given in Table XXI, since an equal number of large and small worms from each genus formed the basis on which these averages were calculated.

Time Required to Leave a Unit Circle. If planarians invariably took a straight radial path in going from the center to the circumference of a circle, the time required to leave a unit circle might be used in computing the rate of locomotion. Such a path, however, is not taken. Nevertheless, records of this kind, although untrustworthy for purposes of accurate calculation, furnish a reliable criterion for the comparison of generic and specific behavior. The differences in behavior in the representatives of three genera and two species are given in Table XXII. *Bdelloura* failed so frequently to emerge from the circle that it is excluded from the list. Each genus and species was tried an equal number of times in light of three different intensities.

As might be expected, Table XXII presents a close parallel to Table XXI. The only difference in the relative values of behavior, expressed by the averages of rate and time in these two tables, appears in the case of *P. maculata*. This species, though first in the scale as regards actual rate of locomotion, is second as regards the time required to leave a unit circle, a condition indicating relatively more wandering on its part than was shown by any of the other worms.

TABLE XXII

The average time in seconds taken in passing from the center to the circumference of a circle 10 cm in. diameter in directive light

	GENERIC DIFFERENCES			SPECIFIC DIFFERENCES	
	Dendro- cœlum	Phagocata	Planaria	Planaria maculata	Planaria gonocephala
Average number of seconds...	62	40	54	47	60
Number of observations.....	120	120	240	120	120

The Effects of Fatigue. To obtain an idea of generic and specific differences in the effects of fatigue, two sets of averages have been combined. First the average rate of ten worms of each kind, when subjected to four successive trials, was first ascertained and the difference between the first and the fourth rate was then expressed as a percentage of increase or decrease in rate, as the case might be. Secondly, the time required to leave a unit circle in twelve successive trials was next recorded and the average percentage of increase or decrease in time of the last four trials, as compared with the first four trials, was computed. By combining these two kinds of percentages the relative differences in the effects of fatigue upon the individuals of the various genera and species, are clearly brought out.

If the results of this computation be compared with the conclusions reached in another way under the preceding paragraphs on "duration of movement," it will be seen that there is a complete agreement in the relative behavior of the different genera and species. That is, the worms most subject to fatigue are the

first to come to rest and those least affected by fatigue continue longest in motion

TABLE XXIII
The average generic and specific difference in fatigue

	GENERIC DIFFERENCES			SPECIFIC DIFFERENCES	
	Dendro- cœlum	Phagocata	Planaria	Planaria maculata	Planaria gonocephala
Percentage of change in rate of the fourth trial as compared with the first.....	-11	+9.5	-9	-13	-5
Percentage of change in time required to leave a unit circle of the last four as compared with the first four of twelve consecutive trials.....	-33	-10	-15.8	-23	-8.7
Average percentage of fatigue	-22	-0.25	-12.4	-18	-6.8
Total number of comparisons	30	20	40	20	20

Responses to Changes in Intensity. When worms in non-directive light pass from a given intensity to one $2\frac{1}{2}$ times as great, decided differences appear in their behavior, the generic differences being plainly of wider range than the specific.

TABLE XXIV
Average differences in response at the critical line separating two areas of non-directive light of which one (82.50 c.m.) is approximately $2\frac{1}{2}$ times as great as the other (33.16 c.m.)

	GENERIC DIFFERENCES			SPECIFIC DIFFERENCES	
	Dendro- cœlum	Phagocata	Planaria	Planaria maculata	Planaria gonocephala
Number of observations.....	45	202	206	50	156
Percentage of responses.....	17	37	52.5	55	50
Percentage of failures to respond.....	83	63	47.5	45	50

In this table Dendrocœlum is shown to respond in only 17 per cent of its passages across the critical line separating the two different intensities of light, while Phagocata responds in 37 per cent

and *Planaria* in 52.5 per cent of the cases. These are differences in degree of response that are great enough to be of unquestionable significance. When, however, *Planaria maculata* and *Planaria gonocephala* are compared in the same way, only a slight difference in the degree of response, namely, that between 55 per cent and 50 per cent, is to be observed.

Manner of Coming to Rest. Although little attention was paid to this point during the series of observations taken up for the present study of planarian behavior, still a few indications of generic difference in the manner of coming to rest appear from the foregoing data. *Bdelloura*, it will be remembered, has a distinctive manner of coming to rest in close rosettes within an area of increased illumination, while *Dendrocœlum* shows a considerable tendency to collect in exclusive companies during periods of inactivity. With regard to the two species of *Planaria* studied, nothing at all definite was observed in this connection which could be called a true specific difference in behavior. Between *Dendrocœlum* and *P. gonocephala*, however, a decided generic difference seems to exist, as several series of records on orientation in directive light show. *Dendrocœlum* according to these records came to rest in an unoriented position in 70 per cent of the cases observed, while *P. gonocephala* failed to take up an oriented resting position in only 59 per cent of the observations. In other words, *P. gonocephala* is more liable to come to rest in a position oriented with reference to the light than *Dendrocœlum*.

Summary. The essential points brought out in the foregoing section are condensed for the sake of brevity and clearness in Table XXV.

In certain instances, namely, in changes in the character of the course (2), the influence of fatigue (7), and the percentage of responses to change in light intensity (8), specific behavior shows a more intimate correlation than generic behavior, otherwise the range between the behavior of *P. gonocephala* and *P. maculata* is greater than the generic differences separating *Planaria* from the other genera under observation.

It might be expected *a priori* that generic differences would exhibit a greater range than specific differences and that similarly,

specific behavior would include more phases of action than individual behavior.

In the present series of records hardly enough representatives of different genera and different species were under consideration to establish any convincing generalization on this point.

TABLE XXV
Comparisons in behavior, generic and specific

CRITERION OF BEHAVIOR	GENERIC DIFFERENCES				SPECIFIC DIFFERENCES	
	Dendro- cælum	Phagocata	Planaria	Bdelloura	Planaria maculata	Planaria gono- cephala
(1) Percentage of negativeness	93.1	89.6	92.2	11	99.9	84.6
(2) Character of course in directive light						
Turns toward the light	without much difficulty	with great indifference	with much difficulty	with ease	No	contrast
Shows goniotaxis	plainly	slightly	slightly	none	slightly	slightly
Wigwag movements	few	few	few	many	few	few
(3) Average duration of movement	18 min.	60+ min.	?	15 min.	47 min.	60+ min.
(4) Amount of wandering (Av. deviation in degrees)	9.4	30.	24.6	39.3	21.6	27.7
(5) Rate of locomotion (In mm. per sec.)	0.85	1.395	1.270	?	1.470	1.075
(6) Seconds required to leave a 10 cm. circle	62	40	54	?	47	60
(7) Comparative influence of fatigue, per cent.	22	0.25	12.4	?	18	6.8
(8) Percentage of response to change in light intensity	17	37	52.5	?	55	50
(9) Manner of coming to rest	in dark in exclusive groups	in dark	in dark	in light in rosettes	in dark	in dark

B Individual Behavior

The analysis of specific behavior leads to the study of individuals, since it is the average activity of different individuals that makes up the behavior typical of the species. In biological litera-

ture animal behavior, particularly among the lower forms, is ordinarily referred to in its specific or even generic aspect. The distinctive actions of individuals, as such, it seems, have usually been outside of the purpose of the observer.⁵

Individuals, however, even among such comparatively simple forms as planarians, do not always act with machine-like uniformity. Until it is possible to predict with exactness what behavior will result under any given set of conditions, an accurate knowledge of the behavior of any kind of organism must be based upon repeated observations of individuals as such rather than as representatives of species and genera.

Individual variations in behavior constantly appeared throughout the course of the present investigation. It will be sufficient, however, for the purpose of making clear their importance to cite only a few instances of such variations.

It should be noticed that whenever "exceptions to the rule" of behavior occur, as in the case of negative planarians coming to rest in the light or becoming positively phototactic for a time (see the two cases cited on p. 78), they are ordinarily simply abnormal cases of individual behavior standing out against the background of average specific or generic behavior. Exceptional cases of this kind, however, are not so typical of what really constitutes individual behavior as the less aberrant actions making up the majority of the movements which the animal performs.

The main point to be recognized, then, is that the individual presents unknown factors, which, even in the simplest forms of life, where the range of variation is least, have never yet been reduced entirely to chemico-physical terms, a fact which impairs somewhat the conclusions of those writers who would draw a complete parallel between an organism and a machine.

Rate on Successive Days. When the rate of locomotion of certain isolated individuals is averaged from four trials, for example, and the same experiment is repeated on the following day with the same individuals, thereby eliminating the effects of fatigue, under as nearly identical conditions as possible, the two sets of figures

⁵Frandsen ('01), who was impressed by individual differences in the phototaxis of *Limax*, and Smith ('02), who worked with the earthworm, are exceptions to this generalization.

thus obtained show more variation than would be expected if the organisms experimented upon responded in a machine-like way. If not all, at least a part of this variation may, then, be due to differences in individual behavior.

TABLE XXVI

The differences among isolated individuals of different species in the average rate of locomotion, based on 4 trials each on each of two different days, expressed in mm. per sec.

	Dendrocœlum lacteum	Phagocata gracilis	Planaria maculata	Planaria gonocephala
First day	1.52	1.22	1.76	0.67
Second day	0.70	0.96	1.11	0.73

The Relative Value of Individual Behavior. In the three following tables individual behavior will be compared with light intensity with respect to (1) rate of locomotion, (2) range of rate and (3) manner of turning.

First, the individual behavior of 10 worms belonging to the species *Planaria gonocephala* under all intensities of non-directive light showed greater range in the rate of locomotion than the average behavior of the same ten worms showed under any single intensity of non-directive light.

TABLE XXVII

The relative effect on rate of locomotion of individual behavior and light intensity. The averages are expressed in mm. per sec.

A VARIATION OF INDIVIDUAL BEHAVIOR

Identification number of worm	1	2	3	4	5	6	7	8	9	10
Average rate in all the intensities given in B.....	0.79	0.57	0.70	0.64	0.83	0.70	0.72	0.60	0.49	0.62

$$\text{Range} = 0.34 [0.83 (\text{No. } 5) - 0.49 (\text{No. } 9)]$$

B VARIATION IN DIFFERENT LIGHT INTENSITIES

Light in candle meters	0	0.94	11	39	78	126	155	217	431
Average rate of the 10 worms given in A	0.57	0.66	0.69	0.75	0.64	0.65	0.69	0.70	0.63

$$\text{Range} = 0.18 [0.75 \text{ '39 c.m.} - 0.570 (0 \text{ c.m.})]$$

Secondly, the range between the maximum and minimum rates of ten individuals in all intensities of non-directive light was greater than the average range of rate of the same individuals under different intensities of non-directive light.

TABLE XXVIII

The relative effect of individual behavior and light intensity on the range of rate of locomotion, expressed in mm. per sec.

A VARIATION OF INDIVIDUAL BEHAVIOR

Identification number of worm.....	1	2	3	4	5	6	7	8	9	10
Maximum rate in all intensities given in B.....	2.58	1.67	2.00	1.67	2.17	2.08	2.20	1.78	1.82	1.58
Minimum rate in all intensities given in B.....	0.92	0.67	0.92	0.83	1.03	0.42	0.67	0.28	0.28	0.75
Range of rate	1.66	1.00	1.08	0.84	1.14	1.66	1.53	1.50	1.54	0.83

$$\text{Range } 0.83 = [1.66 \text{ (No. 1 or 6)} - 0.83 \text{ (No. 10)}]$$

B VARIATION IN DIFFERENT LIGHT INTENSITIES

Light in candle meters	0	0.94	11	39	78	126	155	217	431
Maximum rate for all worms given in A	1.58	1.93	1.92	1.77	1.92	1.79	1.87	2.00	1.55
Minimum rate for all worms given in A.....	0.71	1.07	0.74	1.04	0.77	0.62	1.15	1.03	0.79
Range of rate	0.87	0.86	1.18	0.73	1.15	1.17	0.72	0.97	0.76

$$\text{Range } 0.46 = [1.18 \text{ (11 c.m.)} - 0.72 \text{ (155 c.m.)}]$$

Thirdly, with respect to clockwise or contra-clockwise turnings, individual factors were found to be of more importance than differences of intensity of non-directive light in determining the direction of turning.

It should be added that the ten worms concerned in the three preceding tables were as similar in size and external appearance as it was possible to select.

A Cave Planarian. This specimen came from an Indiana cave, where it probably had always lived in darkness up to the time of its capture. When first made the subject of experiment, it could be briefly described as a white worm, about 6 mm. in

length, devoid of any dark pigment except in the two eye spots. Although most nearly resembling *Dendrocoelum lacteum* in color, it showed some differences from this species in the contour of its body and particularly in its behavior. It was thought probable, therefore, that this was a representative of some species peculiar to a dark habitat. The absence of sexual organs made its exact identification impossible. In the present connection it will be referred to simply as "the cave worm." As a unique subject for the study of individual behavior, it proved to be very

TABLE XXIX

The relative effect of individual behavior and light intensity on the direction of turning, expressed in a ratio of contra-clockwise to clockwise movements

A VARIATION OF INDIVIDUAL BEHAVIOR

Identification number of worm.....	1	2	3	4	5	6	7	8	9	10
Ratio of contra-clockwise to clockwise turn- ings in all the intensities given in B	1 to 1.52	1 to 1.40	1 to 0.42	1 to 4.00	1 to 2.08	1 to 4.21	1 to 2.23	1 to 4.02	1 to 0.85	1 to 0.93
Range = 1 to 3.60 [1 to 4.02 (No. 8) — 1 to 0.42 (No. 3)]										

B VARIATION IN DIFFERENT LIGHT INTENSITIES

Light in candle meters	0	0.94	11	39	78	126	155	217	431
Average ratio of contra-clock- wise to clockwise turnings of the 10 worms given in A...	1 to 1	1 to 1.10	1 to 1.24	1 to 1.65	1 to 1	1 to 0.93	1 to 1.32	1 to 1.40	1 to 1.58
Range = 1 to 0.72 [1 to 1.65 (39 c.m.) — 1 to 0.93 (126 c.m.)]									

interesting. A comparison of its activities with those of other planarians is given in Table XXX, where it will be seen that this cave worm was considerably more active than any other kind of worm under observation, both with respect to locomotion and to the average time required for it to leave a unit circle. Regarding the degree of negativeness which it presented, no new feature appeared, though its average in this point was rather higher than that of all the other worms studied. However, its degree of wandering quite exceeded anything shown by planarians which had been reared in the light.

If the relationship of an animal could be determined by behavior alone, there need be no hesitancy in saying that this unidentified planarian should not be classified under the species *Dendrocœlum lacteum*, since in all the criteria mentioned in the foregoing table it stands at an opposite extreme to *Dendrocœlum*. In point of fact its behavior more closely resembled that of *Phagocata gracilis*, a species which, according to Dr. A. M. Banta, who kindly furnished the cave planarian for this study, is common in the streams in the vicinity of the cave where the latter was found.

TABLE XXX

The behavior of a cave planarian compared with that of planarians accustomed to light.

CRITERIA OF BEHAVIOR	THE CAVE PLANARIAN		OTHER PLANARIANS			
	Number of trials	Average record	Average record	Maxi- mum	Mini- mum	Range
Average amount of negativeness, expressed in the percentage of deviation upon leaving a circle 10 cm. in diameter when started at right angles to incident light	70	99.6	91.8	99.9	84.6	15.3
				(B delloura omitted)		
Average rate in mm. per sec.....	60	2.00	1.203	1.473	0.853	0.62
Average seconds required to leave a circle, 10 cm. in diam.....	90	27.8	52.2	62.2	39.7	22.5
Average deviation in degrees when pointed away from the light. (Amount of wandering)	46	47°	25°·6	39°·3	9°·4	29°·9

Summary. Average individual behavior constitutes typical specific behavior. Variations in individual behavior make accurate predictions of responses to stimuli under given conditions, impossible. The rate of locomotion of the same individuals varies from day to day even under apparently identical conditions. Individual variations in the rate of locomotion, in the range between maximum and minimum rates, and in the percentage of clockwise turnings, are more variable than the average behavior in these particulars under different light intensities.

An unidentified cave planarian showed greater activity and more inclination to wander than any of the other planarians under observation.

(To be continued)

THE REACTIONS OF PLANARIANS TO LIGHT

BY

HERBERT EUGENE WALTER

WITH FOURTEEN FIGURES

(Concluded)

4 BASIS OF BEHAVIOR

In the sections on Photokinesis and Phototaxis certain conditions of illumination were shown to be variable factors in influencing the movements of planarians. Following this treatment of the subject an attempt was made under "Kinds of Behavior" to classify the effects of light according to the way in which the responses of planarians become manifested in a generic, a specific or an individual sense. It now remains to consider the nature of the factors which cause different individuals to present characteristic differences in behavior. There are at least three ways of approaching the matter. These may be roughly indicated as the point of view of the morphologist, the physiologist, and the psychologist.

A Morphological Basis of Behavior

The structure and shape of a planarian, its muscular and ciliary equipment, together with the kind and distribution of its light-receiving apparatus, are some of the morphological factors definitely restricting the kind and range of its reactions to light. These morphological factors may be grouped into, first, those which determine the general form of the body and consequently influence the animal's activities in a broad sense, and, secondly, those directly concerned with the reception of the light stimulus, the photoreceptors.

a General Form of the Body

A normal, full-grown planarian may be expected to give typical reactions to any stimulus. Fragments of a planarian, on the other hand, whether occurring from natural or artificial causes, would not be expected to behave as perfectly developed worms do, and observation shows that they do not.

As previously mentioned, Loeb ('94), and later other investigators, established the fact that planarians with eyes and brain removed are still able to give characteristic reactions to light, while Lillie ('01) found that any fragment capable of regeneration would respond to light.

In all cases of mutilated worms, however, the response to light is slower and less precise than that exhibited by normal individuals, and therefore different in degree if not in character from that of the latter. It has been repeatedly observed that worms mutilated unilaterally perform circus movements regardless of the light. This seems to be a plain case of morphological limitations on the part of the crippled animal, whereby the cilia and musculature of one side, on account of injury, are less efficient than those on the other side. Since it is practically impossible in nature to select at random a dozen planarians of which at least one specimen does not show some sort of mutilation, the modified behavior of morphologically imperfect animals becomes a factor of considerable importance in any general analysis of planarian activities.

Again, with regard to the general form of the body, it seems reasonable to suppose that a mature planarian loaded down with sexual products, or one gorged with food, must encounter mechanical difficulties in responding to light, so far at least as locomotion is concerned, which the same animal when unencumbered would not experience. A few experiments were performed to test this supposition, in which a comparison of the behavior of large and small worms was attempted. Pearl ('03, p. 546), it will be recalled, has shown that in the case of *Planaria maculata*, a worm 11 mm. long travels at a faster rate than one 6 mm. long. This experiment was repeated with a confirmatory result but, as will be seen

upon examining Table XXXI, the same result did not occur when *Dendrocœlum lacteum* was used.

TABLE XXXI

The average rate of locomotion in mm. per sec. of 5 small and 5 large individuals of each of four species, subjected to identical light conditions

SPECIES	LARGE		SMALL	
	Size mm.	Average rate	Size mm.	Average rate
<i>Dendrocœlum lacteum</i> (first trial)	11	0.695	4	1.01
<i>Dendrocœlum lacteum</i> (second trial)	11	0.74	4	0.77
<i>Phagocata gracilis</i>	9	1.58	4	1.21
<i>Planaria maculata</i>	13	1.57	8	1.37
<i>Planaria gonocephala</i>	10	1.17	5	0.98

The worms selected for the experiments detailed in Table XXXI were carefully chosen as to length and did not vary more than a millimeter in any case from the size recorded in the table. The result obtained with *Dendrocœlum* was so unexpected that the same ten individuals were put aside and tried a week later under as nearly identical conditions as possible. As will be seen by the table, the result of the second experiment was in general the same, though not so pronounced, as that obtained in the first set of trials. In the cases of *Phagocata gracilis*, *Planaria gonocephala* and *Planaria maculata*, the larger worms traveled faster than the smaller ones. Why the factor of size should give a different result in the case of *Dendrocœlum lacteum* from that common to the dark-pigmented planarians is by no means clear. It is conceivable that a planarian with undeveloped sexual organs or one whose size was reduced through starvation might have a better ciliary equipment *in proportion to its mass* than a normally adult animal and that in consequence it could travel faster. This supposition explains the behavior of *Dendrocœlum lacteum*, but it does not throw light on that of the other species, of which the smaller individuals, instead of traveling faster than their larger associates, moved at a slower rate. It is possible that in the case of the dark-colored worms reduction in size is accompanied by a

corresponding reduction in the photoreceptive elements, which, according to the experiments of Loeb ('94) and of Parker and Burnett ('00) seem to be in some degree at least distributed over the entire body. If this is true, there would result less stimulation from the light and consequently a slower rate. That *Dendrocœlum lacteum* when reduced in size does not suffer a similar reduction of its photoreceptive apparatus is probable. The work of Lillie ('01), wherein he showed the inability of headless individuals of *Dendrocœlum lacteum* either to regenerate or to respond to light, suggests that the photoreceptive apparatus of this species is not scattered over the entire body, but is rather concentrated anteriorly, in all probability consisting of the eyes only. If this is true, a reduction in the size of the body would not necessarily cause a proportionate reduction in the photoreceptors, and, indeed, the proportion of the light-receiving elements as compared with the mass of the body might increase as the worm became smaller. In this connection it is interesting to note that Gissler ('82) pointed out that in the case of *Bdelloura candida* increasing size of the body is accompanied by a decrease in the size of the eyes, and so far may this inverse ratio be carried that the eyes sometimes disappear entirely in large individuals. If there actually exists some such inverse ratio between the size of the photoreceptors and the mass of the body in the case of *Dendrocœlum lacteum*, it is easy to see why the smaller worms travel faster than the larger ones.

By another series of experiments it was found that the smaller worms of all four species, with the possible exception of *Dendrocœlum lacteum*, orient with less accuracy than the larger worms under the same external conditions. In these experiments, as in the previous ones already described dealing with orientation, each worm was placed at the center of a circle 10 cm. in diameter and headed successively toward, away from, and at right angles in both directions, to the incident light. The average amount of deviation at the circumference of the circle from the direction in which the worms were started, reckoned in degrees, gives a criterion of their accuracy in orientation. The averages of behavior obtained are indicated in Table XXXII.

The lessened accuracy in orientation among the smaller worms, as compared with the larger ones, helps to support the hypothesis that reduction in size entails proportionate reduction in the photoreceptive apparatus. The fact that *Dendrocœlum lacteum* forms an apparent exception to this general rule may also be taken as evidence that in this case the photoreceptive apparatus is more localized than in the other worms studied and conse-

TABLE XXXII

The average deviation (expressed in degrees), at the circumference of a circle 10 cm. in diameter, of large and small worms, each lot consisting of 5 individuals. Each worm was headed successively toward, away from and at right angles in both directions to incident light. The actual sizes of the worms were the same as in Table XXXI

SIZE OF WORMS	DENDROCŒLUM LACTEUM			PLANARIA		Phagocata gracilis	Total average
	First trial	Second trial	Average	maculata	gonocephala		
Large, degrees...	67	70	68.5	57	64	63	63
Small, degrees...	57	85.5	68.5	61	69	72	67.4

quently does not suffer a proportionate decrease when the size of the body becomes less. It is furthermore quite possible that a sexually mature planarian may on that account behave differently in light than an immature one. For instance, Yerkes ('03) states that in the case of the hydromedusa *Gonionemus murbachii*, the sexually mature individuals are the ones most sensitive to light, and Schouteden ('02) found the young of *Daphnia* positive, while the adults were negative to light.

Finally, Harper ('05) has shown that in the case of the earthworm the degree of sensitivity to light depends upon the degree of contraction or expansion of the body, since the photoreceptor cells—which in the earthworm lie interstitially at the bases of the epithelial cells—are more exposed to stimulation when the worm is expanded and conversely more shielded when it is contracted. It is more than likely that planarians offer a parallel instance and that their comparative indifference to light stimulation when in the relaxed resting position is due to the fact that then they present a more rounded contour and consequently their photoreceptors

are more deeply buried from the light than when they are in the expanded position assumed while gliding.

b Photoreceptors

What is the photoreceptive apparatus of the planarian? Is it made up of the eyes only, or partly of nerve-endings or of some special morphological elements homologous perhaps to the photoreceptor cells in the integument of the earthworm as described by Hesse ('96). Or does the central nervous system, the ciliary apparatus, or the musculature receive the stimulus directly without the mediation of special sense organs?

Although these questions were not made the subject of particular investigation in the present study of planarian behavior, certain facts incidentally appear from the observations made for other purposes which bear directly upon these inquiries and may serve as a basis for a brief discussion of the nature and location of the photoreceptive apparatus of planarians. The presence of eyes in the anterior part of the body, together with the wigwag movements which often take place in the same region when a variation occurs in the light conditions, point directly to the conclusion that the anterior end of the worm is more responsive to light than the posterior end. The fact that many planarians continue to react to light with considerable definiteness after the whole anterior end of the body is removed, indicates that this region does not necessarily contain the entire photoreceptive apparatus. Decapitated individuals of the species *Dendrocoelum lacteum*, it should be noted, seem to be exceptional in this respect since, according to Lillie, they fail to react to light.

In further support of the supposition that the anterior end of the planarian is the portion most sensitive to light it was found that the skioptic response of *Bdelloura candida* is confined not only to the anterior end but particularly to the region directly including the eyes. Observations repeatedly showed that if *Bdelloura* was allowed to come to rest in a field illuminated from above only, a sharp narrow shadow thrown across its body produced no visible response unless the shadow included the eyes.

The moment, however, that the eyes were in shadow the worm would elongate and frequently begin strikingly active movements.

It has already been shown (Table XIV, p. 77) that all the different species of planarians upon which experiments were made, traveled at a faster rate when they were started with the anterior end pointed toward directive light than when away from it. A reason may be offered for this characteristic increase in rate on the ground that the anterior end was plainly subjected to stronger stimulation when directed toward the light than when pointed away from the source of the stimulus. In the latter instance it was not only turned away from the source of the stimulus but was shielded also from the light to a considerable extent by the shadow formed by the posterior part of its own body.

Again, when a small beam of sunlight passing through a pin-hole in an opaque screen was directed locally to different parts of a gliding *Planaria maculata*, it was found that tropic response would occur in case one side of the anterior end was illuminated, and that it was not necessary for the eye itself to be included in the illuminated area to obtain such responses. However, when the middle of the body or the posterior end was similarly stimulated the worm could not be made to turn.

From the foregoing observations it seems probable that the photoreceptive apparatus of planarians is mainly but not exclusively located in the anterior end of the body and that considerable specific or generic difference may exist with respect to the extent of the distribution of additional light-receiving organs over other parts of the body. It is interesting to note in passing that Gamble and Keeble ('03) found that in the case of the green rhabdocœle *Convoluta roscoffensis* the sensitiveness to light was at the anterior end of the body only.

Concerning the relative sensitiveness to light of the dorsal and ventral surfaces of planarians, a set of experiments was performed on *Planaria goyocephala* in which the results show an absence of any marked differentiation in this regard. It is well known that in the matter of response to a thigmotactic stimulus the dorsal and ventral surfaces of a planarian show a very striking difference. Indeed, the dorsal surface is negatively thigmotactic to

such a degree that it is practically impossible to make a worm remain with its dorsal surface in contact with any surface, while its ventral surface is just as strongly positive in its thigmotaxis.

In contrasting the receptivity of these two surfaces to light stimulation a field of two adjacent intensities, similar to that used in the experiments on abrupt spacial changes in light intensity (Fig. 3, p. 65), was arranged in such a way that, in the first instance the source of the two lights was below, and in the second above, the field in which the worms were placed. The intensities of the light in each case were approximately 66 and 33 c.m. By this means the responses of the worms could be tabulated as they glided from one intensity of light to another and those given when the light impinged on the dorsal surface compared with similar responses made when the light struck directly on the ventral surface. It will be seen in Table XXXIII that the results do not indicate any particular difference for the dorsal and ventral surfaces with respect to the distribution of the photoreceptors. This condition of affairs, however, may be largely due to the translucency of the planarian's body, which would render light-receiving organs accessible from whatever direction the light primarily comes.

TABLE XXXIII

A comparison of responses made by Planaria gonocephala to a change in light intensity, tabulated with reference to the source of the light and its relative degree of stimulation upon the dorsal and ventral surfaces of the worm respectively

Position of light	Number of observations	Percentage of total responses	Percentage of wigwag movements
Above.....	101	50	42
Below.....	156	53	36

An exact determination of photoreceptors other than the eyes was not made. Both Iijima ('84, p. 438) and Carrière ('82, p. 167) in their histological researches upon planarians found "Neben-äugen" frequent and these occasional accessory eyes have also been described by Jäninchen ('96, p. 259). Such structures may

possibly be interpreted as the connecting link between undifferentiated light-receiving organs and the normal eyes of the planarians.

The part that pigment plays in the reception of light is not as yet clearly defined. It is not probable that pigment in itself constitutes a photoreceptor, though it is usually found associated with sensory cells which are directly concerned with light reception. That it is not an essential factor of a photoreceptor is evident, inasmuch as it is absent from the eyes of albino animals. The secondary rôle of pigment in the reception of light by organisms is admirably pointed out and discussed by Beer ('01).

The presence of pigment in a planarian may, however, modify the animal's response to light stimulation by shielding the sensory cells from light, and since its distribution in general is near the exterior, it may afford some clue to the relative receptivity of internally and externally situated photoreceptors. In other words, if pigmented and non-pigmented worms, for example, exhibited the same behavior in light, it might reasonably be assumed that the photoreceptors were not located internally, since they would be partially shielded from light in the case of the pigmented forms and consequently would give rise to a different response.

It is of interest, therefore, to contrast the behavior of dark-pigmented worms with those in which the dark pigment is absent except in the eyes. This is done in Table XXXIV, but it by no means follows that the contrasts there given between the behavior of dark and light worms are due to the presence of dark pigment in the one case and its absence in the other. Other factors than pigment may very probably have been influential in bringing about variations in the light reactions tabulated. Furthermore, it is inaccurate to refer to a white worm as being non-pigmented, since in that case it would be entirely transparent. The question, then, so far as planarians are concerned, is confined not to differences between pigmented and non-pigmented but to differences between dark-pigmented and light-pigmented forms.

It will be seen from Table XXXIV that when subjected to light stimulation dark-pigmented worms in general show more activity than light-pigmented forms. A single exception to this rule occurred in the case of the cave planarian experimented upon.

This marked difference in behavior might possibly be explained on the hypothesis that the direct effect of light on the deeper lying nervous system is inhibitive; that is, so excessive as to produce a sort of light rigor. Thus the more the central nervous system is shielded from light by pigment the less the inhibitive effect becomes apparent. Certain it is that *Bdelloura candida*, which has dark

TABLE XXXIV

The behavior of dark-pigmented worms contrasted with that of worms not possessing dark pigment distributed over the body. The number of observations in each case is not given since the details of this table have already appeared elsewhere

	DARK PIGMENTED				LIGHT PIGMENTED			
	<i>Planaria maculata</i>	<i>Planaria gonocéphala</i>	<i>Plagocata gracilis</i>	Average	<i>Dendrocoelum lacteum</i>	<i>Bdelloura candida</i>	<i>A cave planarian</i>	Average
Duration of movement in a typical set of experiments, minutes	47	60+	60+	56+	18	15		16+
Percentage of orientation to light upon coming to rest.		41		41	30			30
Wigwag responses at the critical line separating two intensities of non-directive light, per cent		39		39	8.5			8.5
Average number of seconds required to escape from a circle 10 cm. in diameter	48.8	59.6	39.6	49.3	62.2		27.6	44.9
Precision of response								
Deviation in degrees upon emerging from a circle 10 cm. in diameter when headed away from the light.	24.4	25.3	29.1	26.3	10.1		11.4	10.7
Rate of locomotion in mm. per sec.	1.47	1.075	1.395	1.28	0.85			0.85

pigment in its eyes only, may be brought to a standstill very readily by means of light stimulation. With the exception of the eyes it may be possible that the photoreceptive apparatus is not differentiated to such an extent that it could fairly be said that any part of the translucent planarian body is entirely free from the direct stimulation of light. The relation of pigment to light reactions

is, however, by no means settled in the foregoing observations. This matter should be finally tested by comparisons in the behavior of different individuals of the *same species* showing variation in pigmentation or of identical individuals at different times when their phases of pigmentation are unlike, rather than upon individuals of different species.

It has proven impossible to include such a consideration in the present paper, but the preliminary steps toward attempting an analysis of the function of pigment with reference to light reactions have already been made and it is expected that a discussion of this phase of planarian behavior will be presented later. It may be stated here that when *Planaria maculata* is fed with a drop of human blood, a decided increase in pigmentation makes its appearance within a few days, due probably to the oxidation of the hæmoglobin in the blood corpuscles with which the planarians have become gorged. This single observation suggests an experimental means for controlling the amount of pigment in a single individual and it may reasonably be supposed that tests of behavior before and after excessive pigmentation will contribute direct evidence upon the part played by pigment in reactions to light.

Summary. Mutilated planarians in general respond to light with less accuracy than normal individuals.

Small worms move more slowly than large ones in the case of those species whose photoreceptive apparatus is not solely confined to the anterior end of the body. In the case of *Dendrocœlum lacteum*, whose photoreceptive apparatus is relatively greater in small individuals than in large, the rate of locomotion is faster among the smaller than among the larger.

Small worms orient with less accuracy than large ones. Planarians in the relaxed, resting position are less responsive to light than when they are stretched out in the act of gliding, a result probably of the greater exposure of the photoreceptors to light in the latter instance.

The anterior end of the body is the chief photoreceptive region and in certain worms, such as *Dendrocœlum lacteum* and *Bdelloura candida*, the anterior end is apparently the exclusive seat of this function.

No marked difference in response to light is shown between worms stimulated on the ventral surface and those equally stimulated on the dorsal surface.

Aside from the eyes, which form at least a part of the photo-receptive apparatus, no definite light-receiving organs were recognized.

Planarians possessing dark-colored pigment distributed over the body show in general greater activity when subjected to light than forms in which there is no dark pigment except in the eyes.

The central nervous system, as well as the more exterior parts of the planarian, may possibly be stimulated directly by such light as passes through the translucent body.

B The Physiological Basis of Behavior

The continually changing adjustment in any organism between the incoming and the outgoing energy gives rise to varying phases of metabolic balance, which may be designated as different "physiological states." Such physiological states form a noticeable factor in the behavior of any animal, a fact to which Jennings ('04b, p. 109) in particular has called attention.

That the difference between such states is great may be readily demonstrated. A planarian's response to directive light when it is in a relaxed, quiescent condition is plainly different from that exhibited after it has been vigorously disturbed by a brush. In fact, it is extremely difficult to get two animals that are in precisely the same physiological condition, or the same animal in precisely the same state at two different times, since the exact adjustment of physiological states is too delicate a matter to be controlled by the present gross experimental methods.

The attempt is ordinarily made to eliminate from experiments, so far as possible, the disturbing element of changing physiological conditions, that is, to keep constant all the factors except the one which is being subjected to test, and those results are counted as most successful in which such disturbance is reduced to a minimum.

It is the purpose of this section first, to give a possible classification of the different physiological states in which a planarian may

be, and, secondly, to pass briefly in review some of the many ways in which light may change the physiological state of such a worm.

a Classification of Physiological States

It is by no means easy to define even a simple physiological state, since the subtle changes form a continuous series of conditions which pass imperceptibly into each other.

An arbitrary classification for convenience may, however, be made as follows:

- 1 Relaxation, or rest.
- 2 Slight activity, without locomotion.
- 3 Normal activity.
- 4 Violent activity.
- 5 Rigor.
- 6 Exhaustion.

In the first of these states there is a minimum expenditure of energy caused by the ebb of the katabolic processes.

The second and fourth states indicate what are often referred to as conditions of low and high "tonus," but as this term has a technical significance with reference to muscle reactions, it will not be used in this classification. The third state, that of normal activity, is the average condition; it is the most desirable one to maintain in testing the animal's responses to different stimuli. By rigor is understood a state wherein there may be an excessive outgo of energy, but unaccompanied by movement, while under exhaustion is included the condition when energy is not being released because there is none to release.

That excessive or continuous light stimulation may go beyond the point producing rigor or exhaustion and may actually cause death, has been repeatedly proven in the case of bacteria by a long line of observers.⁶ The inhibitive effect of excessive light upon other organisms than bacteria has been pointed out by Berger ('00) with reference to *Cubomedusæ*; by Pearl and Cole

⁶ Tyndall ('78), Downes and Blunt ('77, '78), Jamieson ('82), Duclaux ('85a, '85b, '90), Arloing ('87a, '87b), Roux ('87), Dandrieu ('88), Raum ('89), Pansini ('89), Janowski ('50), Buchner ('92), and Ward ('94).

('02) in the case of various infusoria as well as Hydra, Hyallela, Clepsine, Stichostema and Physa; by Yerkes ('03) for Goniomemus and by Carpenter ('05) for Drosophila.

b Changes in Physiological States Induced by Light

A variety of stimuli besides light may cause an animal to pass from one physiological state to another. For example, the sense of phototaxis was reversed through mechanical stimulation by Towle ('00) in Cypridopsis and by Holmes ('01, '05b) in Orchestia and Ranatra.

The following typical illustration of the manner in which changes from one physiological state to another succeed each other is offered as a basis of comparison with the responses to light itself, which are about to be described. In the absence of mechanical stimuli a planarian may be in a state of relaxation. Very gentle mechanical stimulation causes the worm to lift its anterior end and move it cautiously about, bringing the animal into a state of slight activity without locomotion. If, now, the mechanical stimulus is prolonged or increased in intensity, enough energy is released to put the animal into gliding locomotion, when it may be fairly said to have passed into the state of normal activity. Provided the stimulation is made still more pronounced, the worm can next be forced to forsake gliding for crawling or humping, so passing into the state of violent activity. Further, it is possible by vigorous shaking to throw the worm, temporarily at least, into a condition of inactivity through excessive stimulation, during which the animal would remain quiet, not because it is failing to release any energy, but because it is unable for the time to set free its energy in the form of locomotion. In other words, it is in the state of rigor. Last of all, if mechanical stimulation is repeatedly applied a condition of exhaustion will appear when the worm has no more available energy and so is unable to move at all.

Effect of Different Intensities. As already pointed out, no intensity either of directive or non-directive light was found sufficient to change the condition of normal gliding into crawling.

Moreover, light of any intensity or direction frequently proved ineffective in arousing a quiescent worm into any state of apparent activity, particularly if the worm had but recently passed into the state of rest after a prolonged period of exercise.

Effect of Excessive Light. In the experiments with non-directive light it appeared that *Planaria gonocephala*, when subjected to an intensity of 431 c.m., showed somewhat less activity than at lower intensities, both with respect to rate of locomotion (Table III, p. 57) and to the number of turnings made (Table VI, p. 59); yet, so high a degree of intensity of the light stimulus was apparently not sufficient to cause a change into the physiological state of light rigor. It was comparatively easy, on the other hand, to transform *Bdelloura candida* by means of excessive light from the state of normal activity into that of light rigor.

Effect of Sudden Change in Light Conditions. A sudden change in light intensity either by increase or decrease is more effective in producing a new physiological state than an equal gradual change. The sudden withdrawal of the lamp to a considerable distance, for example, is usually sufficient to throw a worm from a normal state into violent activity, that is, from a gliding movement into a disturbed state in which the anterior end is waved actively about. But if the light is gradually withdrawn the same distance the worm will usually not pass into a different physiological condition.

The sudden introduction of complete darkness was never found sufficient to reduce an active worm more than temporarily to the resting position. Sudden dark might temporarily halt a moving worm, but it would not cause it to come to rest and assume the relaxed contour. In *Bdelloura candida* sudden dark, instead of checking the animal's movements, threw it into violent activity.

Effect of Continued Exposure to Light. Continuous exposure to light results in fatigue, which finally causes planarians to change from the state of normal activity to that of relaxation. The tendency toward such a change is shown in Table XXXV, where the responses of a number of worms newly subjected to light stimulation are contrasted with the responses made by the same worms after they had been moving about for several hours in the light.

The fresh worms show more activity than the fatigued worms do. Otherwise expressed, the worms have a tendency to change into a lowered physiological state upon continued exposure to light.

TABLE XXXV

Fatigue effects due to continuous exposure to non-directive lights forming adjacent fields of different intensities, as shown in the behavior of Phagocata gracilis

RATIO OF THE TWO INTENSITIES	1.96 : 1			13.45 : 1			Average		
	Going into greater intensity	Going into lesser intensity	Average	Going into greater intensity	Going into lesser intensity	Average	Going into greater intensity	Going into lesser intensity	Average
Fresh worms.....	10.5	21	16	45.5	47.5	46.5	28	34.4	31+
Fatigued worms....	2.5	9.5	6	32.5	33.5	33	17.5	21.5	19.5

It may be incidentally noted in Table XXXV that, as has already been pointed out in another connection, the percentage of responses is greater when the contrast between the light intensities is greater, and that both fresh and fatigued worms respond oftener upon going into the lesser intensity than when going into the greater intensity.

The time required for a worm placed in directive light to come to rest; that is, to run the gamut from the state of normal activity to that of rest, becomes gradually shorter with continuous exposure. As fatigue increases the worm shifts down the scale of physiological states in less time than when freshly subjected to directive light. A specific case of this kind has already been described in the paragraph on "duration of movement" (p. 105), where in 39 consecutive trials the change from normal activity to relaxation was first made in 18 minutes, but the thirty-ninth time in $1\frac{3}{4}$ minutes, while the fortieth time even mechanical stimulus failed to arouse the exhausted worm from the resting position.

Effect of Previous Exposure to Dark. Worms kept several hours in complete darkness make a larger percentage of responses to changes in their light environment than those which previous to experimentation have been several hours in light. Individuals removed from the stimulus of light for any consider-

able time are more responsive when subjected to it, for the reason that they are in a physiological state farther removed from fatigue than those worms which have remained a long period in the light. This point is brought out in Table XXXVI.

TABLE XXXVI

Percentage of reactions of two worms, Planaria gonocephala, to a sudden change in light intensity both when previously kept several hours in the dark and also when previously exposed for several hours to light

	Percentage of responses	Number of observations
After several hours in the light.....	54	100
After 48 hours in the dark.....	66	100

Summary. Physiological states grade imperceptibly into each other, but may be tentatively divided into: 1, relaxation; 2, slight activity; 3, normal activity; 4, violent activity; 5, rigor; 6, exhaustion.

Various stimuli besides light may induce a change from one physiological state to another.

No light intensity lower than 431 c.m. is sufficient to throw a worm into a higher state than that expressing normal activity, nor is the absence of light sufficient to bring a planarian to rest.

Excessive light intensity shows a tendency to carry *Planaria gonocephala* from a state of normal activity to one of rigor. *Bdelloura candida* is easily changed into a condition of rigor by light.

A *sudden* change of light intensity acts more immediately than a *gradual* change in causing planarians to pass from one physiological state to another.

Continuous exposure to light induces fatigue, finally resulting in the passage of the worm into a state of continuous relaxation, in which condition it becomes practically indifferent to light. Repeated trials of the time required in constant light to come to rest show that a progressively shorter interval occurs between the state of normal activity and that of relaxation until a point of complete inactivity is reached, the worm finally remaining in the latter state for a prolonged period.

Planarians kept for some time in darkness pass into a state in which they are more responsive to light than individuals exposed for a similar length of time to light.

C Psychological Basis of Behavior

Among the first questions that naturally arise concerning the behavior of planarians in light are those which approach the matter from a psychological point of view. How much can planarians actually see, and can they, by repeated experience, "learn" to adapt themselves to changes in the light surrounding them?

To this kind of inquiry it is most difficult to give a satisfactory answer, for the reason that it is impossible to go beyond conjecture and inference in judging what any animal, aside from man, can see or know or experience. It is only possible to state, in more or less definite terms, the responses which animals make to light, since it is beyond man's power ever to experience how animals "feel" under any circumstances.

a. How Much Can Planarians See?

Broadly speaking it may be said that planarians can distinguish light from darkness. The experiments described on pp. 84, *et seq.*, relating to planarians placed in aquaria so surrounded by backgrounds as to produce regions of different light intensity, point to this conclusion, since when subjected to such differential environments the worms come to rest in the darkened areas.

Again, the numerous responses made at the critical line separating two light intensities may be regarded as evidence of some power of discrimination on the part of the worm between different intensities of light.

It is probable, furthermore, that planarians can distinguish a moving object when that object is of sufficient size and contrasts with its surroundings in its degree of illumination, for the reason that a moving object from which light is reflected, means the same to a worm coming into the vicinity of the object as any other change in the direction of light, such as might be caused by moving a

lamp from one position to another. To changes in directive light planarians are known to respond very definitely, and consequently they may be said to distinguish the motions of objects.

With regard to true seeing, however, in the sense of distinguishing the forms of objects, it is safe to assume that planarians have almost no power whatever, since their eyes are optically unable to form images even if the central nervous system were highly enough developed to interpret images when formed. In the case, therefore, of *Planaria alpina*, which, according to Collin ('91, p. 180), "shuns" *Planaria gonocephala* when the latter has been put into the same aquarium with it, seeking "strenuously to escape" from its larger relative, the conclusion does not necessarily follow that *P. alpina* sees an enemy and experiences the sensation of fear. As previously pointed out (p. 95), the whole matter is probably explainable on the basis of negative chemotaxis alone. To attribute fear, therefore, or any other similar complex sensation, to an organism whose responses are so plainly of a simple reflex nature, is to go quite beyond the evidence.

In the performance of the two great life processes of nutrition and reproduction, light is apparently in no way a direct aid to planarians, since they thrive in situations from which light is entirely excluded, as in caves, and since they habitually frequent places where this factor is reduced to a minimum. Light cannot, then, be regarded as a directly essential factor in the life of planarians.

That light is not essential to the activity of protoplasm has more than once been demonstrated. Engelmann ('79), for example, showed that the streaming protoplasm of plant cells occurs normally in darkness, while Maupas ('87) found ciliates multiplying as rapidly in the dark as in the light.

b Are Planarians Able to "Learn"?

With regard to the ability of these worms to acquire upon repetition an abbreviated form of response; that is, to "learn," a few suggestions may be drawn from experiments already described in other connections.

It will be remembered (p. 93) that when a small aquarium

was delicately mounted upon a turntable, such as is used in "ringing" microscopic slides, a very slight rotation was sufficient to bring to a halt momentarily a gliding worm in this aquarium. It was possible to control this momentary response to such a point of nicety that the anterior end of the worm could be made to halt for an instant without interfering with the onward locomotion of the posterior end. If this slight rotation was repeated at intervals of a second it was found that the worm under observation halted with less and less certainty, until after a dozen or more trials it continued to glide on without halting at all. In ordinary phraseology the worm had learned by experience not to be alarmed by a sudden mechanical shock. The lesson, however, was always very soon forgotten, for after an interval of less than a minute, during which the aquarium remained stationary, the worm responded exactly as it did at first, whenever a slight rotation was made. In a similar way the skioptic response of *Bdelloura candida* became less pronounced upon repetition, until it was possible to throw a shadow upon the animal without obtaining any response at all.

Again, when worms were placed in a field of non-directive light, parts of which were of two different intensities, the number of wigwag responses made at the critical line separating the two intensities grew less after the animals had repeatedly crossed the line. At first the new condition of sharply contrasted light intensities in the worm's field of locomotion called out a large percentage of wigwag responses. Later, however, by repeated experiences the worm became familiar with this feature of its environment and made fewer wigwag motions. A definite instance of such a decrease in response is given in Table XXXVII.

TABLE XXXVII

Responses of Planaria gonocephala on crossing the line separating two intensities of non-directive light

	Wigwag movements	No response	Percentage of response
First 25 crossings.....	21	4	84
Second 25 crossings.....	19	6	76
Third 25 crossings.....	12	13	48
Fourth 25 crossings.....	8	17	32

It will be seen that when *Planaria gonocephala* was first introduced into a field of contrasted intensities, it made the wigwag response at the critical line marking a change of light intensity, in 84 per cent of the first 25 crossings, while during the second, third and fourth sets of 25 crossings, the per cents uniformly decreased until at the fourth 25 crossings the number of wigwag responses fell to 32 per cent. It may be objected that the instances thus far cited in this section find a more reasonable explanation upon the hypothesis of fatigue, but the same surely cannot be said of the following case.

It was found that *Planaria maculata* oriented itself to directive light at successively shorter intervals when the position of the light was suddenly changed. To produce such a series of responses there was placed in the dark room a shallow aquarium with an electric lamp at either end, under the control of the right and left hand, respectively, of the experimenter. A planarian was placed in the middle of the aquarium and the right-hand light turned on. As soon as the worm was fairly oriented to this light and gliding away from it, the right-hand light was turned off and at the same instant the left-hand light turned on. The time in seconds required for the worm to orient to the new light; that is, to turn 180° and begin to glide away, was recorded. On p. 89 a typical series of records of such responses is given, in which the number of seconds required for re-orientation when the source of light was reversed, varied from 260 seconds, at first, irregularly down to 35 upon the sixteenth trial. It will be seen from this series that the worm acquired by experience some degree of facility in adapting itself to certain variations in its environment which it would never be liable to encounter in nature, and that this adaptation cannot be explained as due to fatigue. Davenport and Cannon ('97, p. 32) found similarly that "Daphnias respond more quickly and accurately to light after having made several trips to it."

It is quite certain, however, that any educative attainment which a planarian may experience, or which a planarian may acquire, is exceedingly evanescent and also that there is no evidence that the worm emerges from reflex behavior into responses connected with consciousness.

Summary. The existence of feeling or consciousness among planarians is a matter of pure conjecture.

From their responses it may be inferred that they are able to distinguish dark from light, as well as objects in motion, but it is not clear that they can distinguish the forms of objects.

The knowledge which planarians have of objects in their immediate environment, such as food, enemies, etc., depends largely upon chemical and tactile means. They are, therefore, as well able to go through the entire range of their activities in the dark as in the light.

Upon repetition planarians may in some instances become accustomed to, or acquire greater facility in, responding to stimuli, but this result of experience is almost instantly lost, so that it is doubtful whether these animals possess more than the merest rudiments of the primary criterion of consciousness, namely, the ability to learn.

VI GENERAL CONCLUSIONS

Probably the questions which have occupied the greatest share of attention throughout the literature dealing with the reactions of organisms to light, are the following:

- 1 Is the direction or the intensity of light of more importance in orientation?
- 2 Which theory best explains orientation and phototaxis, the theory of trial and error or that of the tropisms?
- 3 How far is behavior with respect to light, adaptive?

I DIRECTION OR INTENSITY

Before the part played in the behavior of planarians by either the direction or the intensity of light can properly be discussed, it will be necessary to present a brief historical résumé of certain general conclusions reached by investigators along this line.

A Historical

Cohn ('53), Strasburger ('78) and Loeb ('90, '93a) attributed the directive effect of light to the action of the rays. In a later

paper Cohn ('64) abandoned his first position and came to regard intensity as the important element in light, a position also maintained by Famintzin ('67), Engelmann ('83), Oltmanns ('92), Verworn ('01) and even by Loeb ('93b) in the case of *Planaria torva*, which he found came to rest in accordance with the intensity, and regardless of the direction, of the light. Davenport and Cannon ('97) modified this point of view by attempting to show that direction and intensity may each operate independently, producing, respectively, "phototaxis" and "photopathy." Holt and Lee ('01) followed with an excellent summary of the whole controversy, emphatically maintaining, in opposition to Davenport and Cannon, that intensity alone is the only possible operative factor in light stimulation and that direction of the rays has no effect whatsoever except in determining a greater intensity of light with reference to one part of an organism as compared with other parts.

Among more recent investigations Holmes ('03), experimenting with the same organism that led Oltmanns to ascribe the greater importance to intensity, namely, *Volvox*, declares himself in favor of direction, while Zeleny ('05), on the other hand, gives an instance of *Serpulid* larvæ going both toward the source of the light and away from it; that is, moving regardless of direction, in order to arrive in regions of increased intensity.

Carpenter ('05) found that the pomace fly, *Ampelophila drosophila*, will orient to the direction of light after it has first been sufficiently aroused by the intensity of the light, while both Yerkes ('99) and Towle ('00) maintain that direction and intensity are by no means mutually exclusive, and that each may play a part simultaneously in determining the behavior of an organism.

Lastly, it has been made clear by Parker ('03) that, besides direction and intensity of light, the size of the source of illumination may determine the orientation. This theory explains why butterflies alight upon a patch of reflected sunlight which produces a large but faint retinal image instead of flying toward the sun itself, which forms only a small but intense retinal image. In the case of planarians, however, this phase of light stimulation is not operative, since the eyes of these animals are incapable of forming retinal images.

B Conclusions with Reference to Planarians

The behavior of planarians may in general be more satisfactorily explained by regarding, with Loeb, the intensity rather than the direction of the light as the principal operative factor in light reactions. At the same time there is much evidence that the intensity utilized by the organism, is intimately associated with, and powerfully modified by the direction of the light. As a basis for these conclusions the following points will be considered. First, the distinction between direction and intensity; secondly, the way in which directive light modifies the intensity with reference to planarians; thirdly, the action of intensity without the modifying effect of direction, and finally, modifying effects of factors other than light.

a. The Distinction Between Direction and Intensity

Theoretically it is plain that light *per se* with respect to any fixed point, may be regarded in two distinct aspects, namely, that of intensity and that of direction. The intensity of light under ordinary circumstances varies inversely as the square of the *distance* and is independent of the position of the source of light. That is to say, at any points equidistant from its source, light has the same intensity, but the more remote the less is the intensity at any given point. The direction of light, on the contrary, is dependent solely upon the *position* of the source of the light and in no way upon the distance. When intensity and direction are considered with reference not to a fixed point but to an organism presenting three dimensions and made up of differentiated protoplasm, the basis of light relations becomes more complex. Light cannot here be treated as a phenomenon *per se* but must be considered in relation to a differentiating organism.

It is true that intensity in the case of the organism, as in the case of a fixed point, varies with the distance from the source of the light. A decided difference, however, appears in the case of the organism inasmuch as, owing to its structure, *the intensity received by it varies also in accordance with the position of the light.* This

second form of variation in intensity is directly due to the fact that the organism has a solid form and is not homogeneously photo-receptive.

The direction of light with reference to the organism, presenting as the latter does a structurally diversified form, is influential only as regards the position of the source of light, just as in the case of a fixed point.

Any change in the position of the source results, then, in a redistribution of the intensities falling upon the organism, so that again the intensity received varies in accordance with the position of the light.

It is this factor of *position* in light that has been termed the directive influence of light and it is seen to be due to variations in the intensity of light with reference to the organism, and not to any peculiar property of light itself. By "non-directive light," on the other hand, is understood those conditions which secure for the organism equalized or symmetrical intensity with respect to the parts stimulated. If this interpretation is correct there can be no response, strictly speaking, to the *direction* of light *exclusive of intensity* although the factor of intensity may be continually modified by that of direction in the light relations of organisms.

b The Modifying Influence of Direction

It is undeniable that the planarians experimented upon exhibited without exception a definite characteristic phototaxis, that is to say, they habitually go either toward or away from the source of light according as they are respectively positive or negative. In analyzing this phototaxis it seemed desirable to eliminate so far as possible the factor of intensity, but the attempt to do this was only partially successful owing to physical limitations. A step was made, however, toward subjecting worms to directive light without at the same time exposing them to a variation in intensity by inserting a biconvex lens between the source of the illumination and the aquarium, thus making the diverging rays of light parallel throughout their course in the aquarium. By this means was formed a field equal in its amount of illumination at

the two ends of the aquarium, the one opposite and the one next to the source of light, with the exception that there was a slight difference at the two ends due to the fact that light in its passage through water is partially absorbed. But modification of light in any degree results in producing less intensity at the farther end of the aquarium, though this difference is less pronounced when a lens is employed. Therefore, although worms placed in this apparatus went with considerable precision in the direction of the propagation of the light, there is no certainty that their behavior was not due simply to differences in intensity. Worms which are thus apparently traveling directly in accordance with the direction of the light, are meantime being subjected to different intensities at the anterior and posterior ends of the body, for the reason that the anterior end is more or less shadowed by the rest of the body, since the latter cuts out a certain portion of the light received at the posterior end.

That direction of light is a factor by no means to be disregarded, even if it cannot be proven to be the immediate cause of phototaxis, is apparent when it is recalled that slight changes in direction call out corresponding changes in the course of the gliding planarian, whereas considerable changes in intensity when the direction remains constant and particularly when such changes are gradually made, may fail entirely to produce corresponding changes in the worm's behavior. This is due to the fact that slight changes in direction may cause considerable changes in the asymmetry of illumination. When a worm, for example, is receiving horizontal light from behind, its head is more or less in shadow, the sides of its body being at the same time equally illuminated. The moment the light is shifted in even a small degree to one side, one entire side of the animal may receive an increase of illumination and the opposite side be thrown into shadow. Thus a slight change in position initiates a fundamental change in the distribution of intensity over the planarian's body.

c Instances of Behavior Due to Intensity Alone

The effect of intensity as a separate factor from the directive influence of light is clearly demonstrable in certain phases of

light reactions. To isolate intensity by excluding the possibility of directive light; that is, to secure equalized intensity with reference to the organism, is not difficult and the manner in which this was done, with non-directive light falling upon a horizontal field from above, has been sufficiently detailed in the body of the paper.

It may be briefly recalled that planarians experimented upon by this method showed a certain unmistakable degree of response which could be referable only to differences in equalized intensity. For example, the rate of locomotion was found to be faster in any non-directive intensity up to 431 c.m. than in darkness, although light in itself was not always sufficient to start a worm into activity, nor was its absence sufficient to check an animal already in motion. Again, though no close correlation between behavior and the degree of intensity was found to exist, there appeared certain general results which were plainly referable to intensity differences only. Instances of such results are the behavior of *Planaria gonocephala* (which was modified in several particulars at 431 c.m. as compared with its behavior at lower intensities); the coming to rest in regions of diminished intensity of individuals of all species except *Bdelloura*; and the increase of wigwag responses corresponding to an increase of intensity differences when a field of contrasted intensities was used.

It is interesting to observe that increase in the intensity of non-directive light, and continued exposure to non-directive light of constant intensity, both tend to produce the same behavior that would result in directive light. Under any of the three conditions just mentioned there resulted by actual experiment fewer turnings, fewer "indefinite changes" and more nearly straight paths on the part of planarians than occurred when the worms were (1) placed in non-directive light of lower intensity, (2) subjected a short time to non-directive light of constant intensity, or (3) left in darkness. Now, fewer turnings, fewer "indefinite changes," and more nearly straight paths are ordinarily characteristic results of directive light, so that here is a case of reactions, which if resulting from the employment of directive light would be termed phototaxis, occurring in non-directive light as the result of intensity alone. Mast ('03) experimenting upon the reactions of planarians to thermal

stimuli obtained a similar result. He observed that apparently "negative" as well as "positive" responses resulted when the animals were subjected to non-directive thermal stimuli.

Another noticeable phenomenon with reference to responses to intensity is, that more wigwag responses occurred at the critical line separating two different intensities when the lesser of the two intensities was 16 c.m. than when it was 33 c.m. (Table XI, p. 69). Similarly responses were more frequent when planarians were subjected suddenly to dark than when they were flooded suddenly by light, and, throughout a large number of series, responses were invariably more frequent when the worms were passing into a region of diminished intensity than when they were entering an area of increased intensity. It is to be inferred that all these phases of behavior are due to the probable fact that the lower intensities compared are nearer the worm's optimum as regards light than the higher ones, since the latter apparently have a tendency to inhibit activity.

Lastly, the relative part played by intensity of light varies decidedly in different species of planarians. The relative intensity in different parts of an aquarium, when no lens is used to lessen the contrast, has comparatively little influence upon *Phagocata gracilis*, as its extensive wanderings (typically reproduced in Fig. 11) toward and away from the source of light, indicate. *Planaria maculata* and *Planaria gonocephala*, on the contrary (Figs. 13 and 14), notwithstanding their ability to come toward the light in the direction of the "rays" throughout the farther half of the dish, seemed invariably to encounter an impassable barrier as soon as they approached within a certain intensity, thereby showing a more delicate responsiveness to intensity differences.

d The Modifying Effect of Other Factors

In attempting to analyze the relative bearing of the intensity and of the direction of light upon the behavior of planarians there must be constantly kept in mind two general sources of error which are always present when these factors of light are in operation. These

are (1) the physiological state of the organism at the time of observation, and (2) the simultaneous effect of other stimuli.

A physiological state may be directly traceable to known causes, such as previous exposure to other stimuli or the condition of metabolic balance in which the animal chances to be at the time of observation, or, again, it may be the result of factors at present unknown, which consequently, although in active operation, are not susceptible of analysis. In any case it is certain that the uncontrolled factors comprehended under the term "physiological state" prove individual planarians to be not identical mechanisms, but organisms possessing a more or less definite individuality. Moreover, it has been shown that differences in physiological state play a greater part in the determination of behavior than do intensity differences in the light stimulus. When a planarian is approaching a state of fatigue, for example, it becomes indifferent to differences of intensity.

With regard to the simultaneous effect of other stimuli acting in conjunction with light, it has already been pointed out that behavior is the resultant of all the factors, external as well as internal, which may be acting upon an organism at a given time, and that consequently the effect of any one of the operating factors, such as that of light, for example, cannot be determined unless the value of the other factors involved is also taken into account. In support of this view, which is so self-evident, it will be recalled that some of the ways in which the responses of planarians to light may be modified by geotaxis, thigmotaxis, goniotaxis and chemotaxis, were touched upon.

Summary. Direction and intensity are separable qualities of light. Direction is dependent upon the relative positions of the light and the organism, whereas intensity depends upon the distance between the light and the organism as well as the initial intensity of the light.

When applied to living organisms intensity may act independently of direction, or in conjunction with it. Direction cannot act independently of intensity upon organisms, since the latter possess definite form and consequently cannot receive the light at a single point.

With reference to an organism, directive light is resolvable into unequalized intensity and non-directive light into equalized intensity.

Asymmetrical intensity in directive light is largely due to the partial shadowing of that part of the body farthest away from the source of the light. Slight changes in the position or direction of the light may cause considerable changes in the symmetry and the degree of the shadow effects and consequently in the relative intensity of the light on different regions of the body of an organism.

To different degrees of equalized or symmetrical intensity planarians show considerable response, but the correlation between their behavior and the degree of intensity is not so close as it is in the case of asymmetrical intensity.

Increase in intensity of non-directive light, continued exposure to non-directive light of constant intensity, and change from darkness to non-directive light, all tend to bring about apparent phototaxis similar to that occurring in directive light.

Responses are more frequent on the part of planarians in intensities approaching the optimum than in higher intensities, where there is a tendency to inhibition.

Relative differences in responses to various intensities are due to specific differences between planarians.

The physiological state of an organism together with the influence of known stimuli other than light are constant sources of error in estimating reactions to light. These factors taken together play a more important part in planarian behavior than light stimulus.

Finally, the action of light upon planarians is a function of its intensity, which, under certain conditions, is emphasized by the direction of the light.

2 TRIAL AND ERROR OR TROPISM?

It is apparent from the preceding section that light may have two effects upon organisms. Of these, one is a kinetic effect, arising from the intensity of the stimulus and resulting in a gen-

eral activity termed photokinesis, while the other, connected indirectly at least with the direction from which light impinges upon an organism, is called phototaxis. In the case of planarians these two phases of light stimulation have been shown to be intimately associated and both operative. Carpenter ('05) pointed out in the case of the pomace fly that phototaxis occurs only when preceded by photokinesis or some other reaction, and such an interrelation of the two is undoubtedly of wide occurrence. The object of this section is to inquire into the causes underlying phototaxis. Loeb ('93b) has shown that phototaxis is the result of orientation. It does not necessarily follow, however, that orientation invariably results in phototaxis. In fact Dearborn ('00) found that crayfishes would orient to an electric light introduced into the water near them without making any considerable locomotor movements in consequence.⁷

To the question of how orientation of organisms to light is caused, three possible explanations may be presented: 1, Chance result of photokinesis; 2, reflex response to directive stimuli; 3, voluntary action. Since the first hypothesis seems entirely inadequate to account for the uniformity of orientation in planarians, and the third alternative is out of the question with reference to these animals, a consideration of the reflex responses to directive stimuli may be taken up at once.

There are two general theories which attempt to explain the way in which orientation occurs through reflex responses to stimuli. These theories are first, the *trial and error theory* of Jennings and Holmes, and secondly, the *tropism theory* of Verworn and Loeb. By the trial and error theory orientation, with its consequent phototaxis, is interpreted as the result of repeated attempts on the part of an organism to become adjusted to any given stimulus. Those attempts which fail to result in adjustment to the stimulus are "errors," and as such are followed by other attempts until finally some one secures the necessary adjustment. Trials of this kind may be made in different ways according to the organism

⁷ Throughout the following discussion orientation will be understood as a *position* assumed with reference to the light while phototaxis will be made to include motion *toward* or *away* from the source of the light.

in question. Among the infusoria and rotifera, as Jennings has shown in a masterly series of papers,⁸ such attempts at orientation are made by means of a "motor reflex," consisting in (1) a sudden withdrawal from the stimulus, (2) a rotation toward a structurally defined side of the asymmetrical organism, and (3), lastly, an advance in a new direction.

In the case of organisms which do not possess marked asymmetry the trial and error method, as pointed out by Holmes ('05a), resolves itself into a series of "random movements;" that is, a number of apparently experimental movements are made, which finally result in the best adjustment to the stimulus.

In both of these methods the organism acts as a unit and not in response to localized stimulation received asymmetrically.

The tropism theory, on the contrary, is based upon asymmetrical action as the result of asymmetrical stimulation. If an organism receives a stronger stimulus on one side of its body than on the other, the result, whether direct or indirect, is that it moves in such a way that this asymmetrical stimulation becomes symmetrical. In other words, orientation occurs.

It is unfortunate that the tropism theory was made to apply to the behavior of the infusoria, since it has been shown beyond doubt by Jennings that exact observation of the behavior of these organisms and an analysis of its details does not admit of the tropic interpretation, but is, on the other hand, explained by the trial and error theory of motor reflexes. It is also to be regretted that the unquestionable rout of the tropism theory, as applied to certain protozoa and other asymmetrical forms, should have led to an attempt to exclude it from the remainder of the animal kingdom.

In a paper on the tropism theory Jennings ('04a) names as an essential criterion of tropism the direct unilateral stimulation of the motor organs. After showing how inadequate such an assumption is to explain the orientation of animals, *particularly that of Infusoria*, he continues ('04a, p. 104), "We should perhaps con-

⁸ See bibliography in Contributions to the Study of the Behavior of Lower Organisms. Carnegie Inst. of Washington. Publication No. 16. 256 pp. 1904.

sider here a modification of the original form of the tropism theory that has been proposed by some authors. This is in regard to the assumption that the stimulating agent acts directly on the motor organs upon which it impinges. For this it is sometimes proposed to substitute the view that the action of the stimulating agent is directly on the sense organs of the side on which the stimulus impinges and only indirectly on the motor organs through their nervous connection with the sense organs. When thus modified the theory of course loses its simplicity and its direct explaining power, which made it so attractive. In order to retain any of its value for explaining the movements of organisms, it would have to hold at least that the connections between the sense organs and the motor organs are of a perfectly definite character so that when a certain sense organ is stimulated a certain motor organ moves in a certain way. When we find, as we do in the flatworm (see the following paper), that to the same stimulus on the same part of the body, under the same external conditions the animal reacts sometimes in one way, sometimes in another, the tropism theory, of course, fails to supply a determining factor for the behavior."

It seems to me that the mechanism by means of which the asymmetrical response is brought about is immaterial, so long as that response can be shown to be the result of asymmetrical stimulation. Asymmetrical response might occur either from direct stimulation of the motor organs as was implied in the earlier papers on the infusoria, or by means of a more complex method, consisting of stimulation of the sense organ, transmission to the central nervous system and thence to the motor organs.

The outcome in either case would fulfill the demands of the tropism theory, if asymmetrical response to asymmetrical stimulation be taken as its criterion. In the quotation just cited, the objection that such transmission compels stereotyped behavior is hardly valid, since stereotyped reaction is by no means the only alternative of asymmetrical stimulation. That flatworms do not respond uniformly to directive stimuli cannot be disputed, but that fact does not exclude the possibility of all tropic reaction on their part. The imperfection of response may be simply the result of imper-

fections in the worm's nervous circuit, assuming that planarian reactions are due to indirect rather than to direct stimulation of the motor organs. In fact, repeated evidence of the failure of a constant and perfectly invariable orientation on the part of planarians has been given in the preceding pages. Such failure, moreover, is quite as likely to occur in the application of the tropism theory to behavior as it is in the case of the trial and error theory, since stereotyped reactions and forced movements, as Holmes ('05a, p. 112) has emphasized, are no more characteristic of tropisms, which depend upon a differentiated stimulation and response, than they are of trial and error movements, resulting from a single motor reflex given in response to all kinds of stimulation.

Furthermore, it has been urged that tropism indicates a simpler form of reaction than trial and error for the reason that it involves only a local part of an organism while the motor reflex of trial and error requires that the organism act as a whole. Consequently, since motor reflex has been indisputably demonstrated as the method of infusorian phototaxis, Jennings ('04a, p. 95) asks, "Should we conclude that the reactions in the higher metazoa are simpler and less unified than in the protozoa?"

That the motor reflex, which occurs with machine-like uniformity, regardless of the point where the stimulation is received, is more complex in character than the stimulation of an asymmetrical part of an organism which *may* depend for its response upon sense-organ, nervous transmission and motor apparatus is an assumption difficult to sustain. It seems more reasonable to agree with Harper ('05) in placing tropism higher in the evolutionary scale than trial and error.

The fallacy that "tropism leads nowhere; it is a fixed final thing like a crystal" (Jennings, '04c, p. 251), while trial and error alone offers possibilities of the higher evolution of phototaxis, has already been answered by Holmes, who points out that trial and error, at least that phase of trial and error depending upon motor reflex, is even more fixed and stereotyped than the reactions occurring in accordance with the tropism schema. To quote: "The end result of both methods is the same, *i. e.*, to get the organism away

from the stimulus. In the one case it is accomplished by direct reflex without more ado; in the other, only after a considerable waste of energy in inconsequential vermiculations" (Holmes, '05a, p. 110).

It is at least conceivable that under the tropism schema, as the nervous differentiation of an animal becomes more complete, the ability of the organism to interfere with and modify its machine-like responses to external stimuli might also increase, resulting in a flexibility of behavior which would present quite as much variation for natural selection to act upon as that evolved by the trial and error method. This point of view by no means denies that trial and error is the usual "method of intelligence" (C. L. Morgan '00, p. 139). It is simply an attempt to recognize in the method of tropism also one of the possibilities of evolutionary progress in behavior and as such holding a higher position in the scale of evolutionary methods than trial and error by motor reflexes.

It has been shown (p. 143) that planarian responses of an apparently asymmetrical character may occur as a result of symmetrical stimulation. Similar instances in the case of planarians have also been demonstrated by Mast ('03) with reference to thermal stimuli. This, however, is no exception to the validity of the tropism theory, in which asymmetrical responses result from asymmetrical stimulation. Because a planarian *may* make an apparently phototropic response when subjected to symmetrical stimulation, is not evidence against the supposition that the usual phototropic response is due to asymmetrical stimulation.

The "wigwag" movements of planarians, to which repeated reference has been made in the preceding pages, resemble superficially the "random movements" of the earthworm as described by Holmes. They do not, however, ordinarily appear to be the basis of trial and error selection resulting in orientation, since in a majority of cases, after a worm halts and makes wigwag movements it continues on its way without a change of direction. The movements of *Bdelloura candida*, as shown in Fig. 12 form an exception to ordinary planarian behavior in this respect.

As a rule wigwag movements are probably occasioned by a general disturbance arising from some stimulation which throws

the worm into a different physiological state. Exploring movements, such as these seem to be, may bring about asymmetrical stimulation, in which case the worm makes a tropic response.

It was particularly noticed that when planarians received light from below, the anterior end of the body was frequently tilted back and forth as if to make it possible for the light when coming from such an unusual direction to enter the pigment cups of the eyes. The phenomenon suggested the craning of necks and bobbing of heads among a crowd of people who are all trying to see the same object at once.

Wigwag movements seem to be oftener connected with changes in the intensity of light than with changes in its direction. When the latter occur, tropic response is immediately the result.

In the course of the experiments previously described wherein the worms glided from an area of one intensity of non-directive light into another it was noticed that in a majority of cases when the critical line was not crossed at right angles, no change in course occurred, even when the worm halted and made wigwag movements. Of course at a certain instant of any diagonal crossing of the critical line one eye must receive more stimulus than the other, in which case according to an inflexible tropism theory asymmetrical response ought to occur. But such a response does not frequently appear and the reason for this becomes clear when it is remembered that a considerable number of responses were shown to occur which were called "latent wigwags" (Fig. 4, *E*), because they failed to make their appearance until in some instances the worm had passed more than the length of its body beyond the critical line. Since, therefore, latency of response to intensity is by no means uncommon, it is evident that the brief interval of asymmetrical stimulation occurring when a worm glides diagonally into an area of different intensity is not sufficient to result in an asymmetrical response.

Two conclusions, then, seem reasonable, namely, that phototaxis as related to planarians is primarily due to asymmetrical response resulting from asymmetrical stimulation, and that wigwag movements, together with similar apparent trial and error forms of behavior, contribute chiefly to this end, *i. e.*, to phototaxis.

Summary. Orientation may occur without phototaxis.

Two theories have been advanced to explain orientation and phototaxis in lower organisms, namely, the trial and error theory and that of the tropisms. The former may be based upon "motor reflexes" or upon "random movements" according to the symmetry of the animal.

The tropism theory rests upon asymmetrical response to asymmetrical stimulation. It does not necessarily depend upon the direct stimulation of the motor organs, nor is it essentially stereotyped in its character any more than are trial and error responses by motor reflex or random movements.

The tropic form of response may, and probably does, require a more complex mechanism than that which causes the motor reflex, consequently it is the form of response to be logically expected among planarians, since the motor reflex has been proven to be the form utilized by the protozoa.

Tropisms, as well as trial and error movements, provide, through the modifying control of an evolving central nervous system, sufficient latitude of variation for natural selection to work upon in the evolution of higher forms of behavior.

Asymmetrical response may, in certain cases, result from symmetrical stimulation, but ordinarily its cause is asymmetrical stimulation.

Wigwag movements are occasioned most frequently by changes in intensity, and they may result in orientation and phototaxis by assisting an organism to secure asymmetrical stimulation.

Latency of reaction accounts for some of the failures in orientation which often occur even when asymmetrical stimulation is acting upon an organism.

Finally, the orientation and phototaxis of planarians is more consistently explained by the theory of tropisms than by the theory of trial and error.

3 ADAPTATION.

It remains, finally, to inquire how far the reactions of planarians to light are adaptive; that is, how far the response to light is "of

such a kind that it better insures the existence of the individual, or of the race" (T. H. Morgan '03, p. 1).

It is evident that the generally negative character of the reactions of planarians to light indicates a tendency on the part of these worms to reduce as much as possible the amount of light stimulation received or to avoid it altogether. The rigor effects of excessive stimulation furnish evidence also that light is a factor in a planarian's environment which it finds unavoidable and unwelcome and to which it is adapted only in a negative fashion. In fact the vague distinction separating "lower" from "higher" animals consists largely in the ability of higher animals to assume an active aggressive rather than a passive defensive relation toward the factors making up their environment. For example, the evolution in animals of the visual organs, which in the planarians is only inceptive, enlarges the possible range of photic responses until light becomes an essential factor in an animal's environment, contributing largely to its welfare by enabling it to see its food, to avoid its enemies and to select its mates. It is plain that light plays no such important part in the activities of planarians, for, as has already been pointed out, light *per se* is not essential to planarians, since they are known to live successfully in dark caves. Moreover, so far as known, light does not influence the regenerative or reproductive processes of planarians in any way whatsoever. The formation of pigment may perhaps be regarded as an adaptation to light conditions, inasmuch as animals possessing pigment are thereby shielded to a certain degree from excessive stimulation.

With reference to activities connected with nutrition and reproduction, planarians are not dependent upon light stimulation. They are otherwise equipped, since they doubtless find their food by chemotactic means and avoid whatever enemies they may have, not aggressively nor actively by retreating from visible foes but rather in a passive way by remaining concealed from enemies that might see them. They have no organs of defense but survive by escaping attention. In this sense their negative phototaxis may be regarded as of protective value and consequently adaptive.

Furthermore, the geographical distribution of fresh water pla-

narians has been shown by Borelli ('93) and Wilhelmi ('04) to be chiefly dependent upon temperature and almost not at all upon the amount of illumination to which they are subjected. Voigt ('04) noticed that worms when hungry may be seen wandering about even in patches of bright sunlight with apparent disregard of light. This seems to be a case of the light reactions becoming over-balanced by other responses.

Summary. Light is not an essential factor in planarian activities, since the behavior necessary to the welfare of the individual and the race is mainly referable to other factors.

A planarian's response to light is of a passive character, which may have an adaptive significance only in so far as its phototaxis tends to conceal the worm from its enemies. The presence of pigment may also be regarded as an adaptive condition induced by the animal's relation to light.

The evolution of the photoreceptive apparatus of the planarian has not reached the degree of differentiation necessary to enable it to secure for itself such adaptations to the factor of light in its environment as would make aggressive activity possible to it in a manner characteristic of higher animals.

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REGENERATION IN COMPOUND EYES OF CRUSTACEA

BY

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WITH SIXTEEN PLATES AND TWO FIGURES IN THE TEXT

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I INTRODUCTION

The regeneration of the Crustacean compound eye has been made a subject of observation by a number of investigators. But for the most part end results alone have been described. Very few details of the processes involved in regeneration have been given. To give an accurate description of the histogenesis of the regenerated structures is the chief aim of this paper in the belief that it will contribute something toward a more accurate understanding of the more general problem of regeneration.

The problem has separated itself into three main divisions: first, the regeneration of a functional eye; second, a search for the causes of no regeneration and abnormal regeneration, and third, observations upon the heteromorphic regeneration which may follow the removal of the entire eye.

Herbst ('96, '00) and Morgan ('98) have made the principal observations upon the regeneration of the Crustacean compound eye. But so far as the particular phases dealt with in this paper are concerned Herbst's descriptions are not sufficiently detailed to be of especial assistance in this work. Herbst's observations upon the regenerated heteromorphic structures are somewhat more extensive and in some respects furnish an excellent basis for comparison with the results to be discussed in this paper. For the most part, however, where results have been compared with the work of others the comparison is made between the regenerative and embryonic development and between normal and regenerated structures.

The terminology employed is in great part that used by Parker ('91) in his work upon the compound eyes of Crustacea, it being the terminology in most general use. A few minor deviations have been made but no wholly new terms have been introduced.

The following plan has been followed in this paper. First, a brief description of the normal adult eye is given to furnish a basis for comparison. This is followed by a description of the preparatory regenerative processes which in reality constitutes one of the most important phases of the subject. Then the regenerative processes proper are described under the three main divisions already suggested.

The experimental part of the work was begun at the University of Pennsylvania. Observations upon living material were also carried on during two summers at Woods Hole, Mass., and one summer at Cold Spring Harbor, L. I. The greater part of the detail work of examining the preserved material has been done at the University of Missouri during the present year.

In closing I wish to express my thanks to Dr. E. F. Phillips and Dr. D. B. Casteel for the care of experiments; to Dr. E. G. Conklin, University of Pennsylvania; Dr. C. B. Davenport, Carnegie Institute at Cold Spring Harbor, and Dr. George Lefevre and Dr. W. G. Curtis, of the University of Missouri, for their interest and valuable suggestions during the course of the work.

II MATERIAL AND METHODS

The small hermit crab, *Eupagurus longicarpus*, the common shrimp, *Palæmonetes vulgaris*, and the sand shrimp, *Crangon vulgaris*, afford the greater part of the material used in the series of experiments to be described in this paper. For comparison two species of crayfish, *Cambarus virilis* and *C. gracilis*, a species of fresh water *Ascellus*, the common (wood-louse), *Oniscus*, and the fresh water *Gammarus* were used. Other Crustacea also were experimented upon but since no decisive results were obtained they need not be considered here.

The work has been confined chiefly to the eyes of the forms used although experiments upon the appendages, particularly the antennæ, were conducted at the same time. These were, however, largely for the purpose of comparing relative rates of regeneration of the different parts, especially the rate of regeneration of the appendages as compared with that of the eyes.

The experiments upon the eyes consisted in either the removal of a part of the eye or of the whole eye. The part removed varied greatly in the different series of experiments and more or less in individuals of the same series. A limited number of experiments upon *Palæmonetes* included the removal of both eyes or the removal of one eye with a part of the brain; these operations in most cases resulted fatally. The effect of splitting the eye was

also tried upon two series of *Palæmonetes*, each series was composed of a considerable number of individuals. Results, however, were not particularly different from those obtained after removing a part of the eye.

A total of 600 *Palæmonetes* and hermit crabs had either one or both eyes operated upon. A much smaller number of Crangon and crayfish were used. No accurate account of the *Ascellus*, *Oniscus* and *Gammarus* was kept. More than 50 per cent of the *Palæmonetes* and hermit crabs died immediately, or within a short time, after the operation, many of them dying within a few minutes after the eye was injured. Of the survivors about 58 per cent lived through one or more moults.

Forty-two Crangon had the eye operated upon and of these one died of the operation. The crayfish used were for the most part *C. gracilis*, measuring from 12 to 15 mm. in length, that had moulted but once after hatching.

Palæmonetes and Crangon moult once about every ten days to three weeks. The hermit crabs moult much less frequently, often but once in two or three months. The hermit crabs regenerate, however, as rapidly as *Palæmonetes* or Crangon.

Considerable difficulty was experienced in keeping the animals alive and in keeping individual records. Finally the plan of keeping each animal in a separate finger bowl was adopted. This method was fairly satisfactory except in very warm weather. Then the water became warm and unless it was changed often the animals soon died. Chopped-up bits of clam were fed to them two or three times a week. Great care had to be used in warm weather for the water became foul, if the food was left in more than three or four hours, and caused the death of the animals.

In spite of all precautions various accidents occurred which resulted in the death of promising material. Twice attempts were made to transfer the experiments from Philadelphia to Woods Hole or vice versa with disastrous results in each case. The failure was due in part no doubt to the extreme warm weather. For although every known precaution was taken most of the animals died within twenty-four hours.

A number of the ordinary fixing fluids were used to preserve

material. Among those most frequently used were Fleming's osmic fixative, Perenyi's fluid, Kleinenberg's picro-sulphuric, picro-aceto-sulphuric, Petrunkevitch's fluid and alcohol acetic. Other fluids also were used and boiling water was tried. The best results were obtained from picro-aceto-sulphuric and Perenyi's fluid. In any case it is difficult to obtain a fixative that does not shrink the inner tissues from the chitin, the regenerated tissues being much more easily affected in this respect than normal tissues.

The embedding was done altogether in hard paraffine 54° to 58° C. melting point. It was necessary to embed the material for a long time in order to cut it without tearing the chitin from the softer tissues. The most satisfactory infiltration was obtained by placing the objects first in equal parts of oil and paraffine, leaving them on top of the water bath over night and then replacing the oil and paraffine with pure paraffine, leaving them on top of the water bath from eight to ten hours longer. Finally they were put in the paraffine bath from one to two hours. Even after the most thorough infiltration it was well nigh impossible to obtain complete series of good sections, because of the difficulty of cutting through the different textures of the material. In dehydrating preparatory to embedding, cedar or bergamot oil was used in preference to xylol as these oils made the tissues less brittle.

The chief stains employed were Fleming's triple stain and Heidenhain's iron hæmatoxylin. Various counter stains were used with the iron hæmatoxylin but the most generally satisfactory were acid fuchsin and orange G.

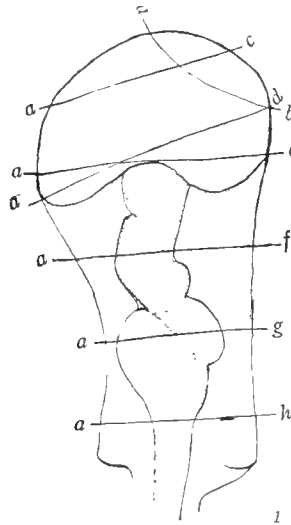
In most instances no attempt was made either to soften the chitin or to remove the pigment before the eyes were sectioned. As a rule, however, the material was fixed shortly after a moult so that the chitin was as soft as could be obtained. Any sort of a reagent used to soften the chitin seemed to be more or less injurious to the softer parts, particularly the regenerating tissue. When the chitin was removed, however, 2 per cent nitric acid in 70 per cent alcohol was found to be most satisfactory. It was generally disadvantageous to remove the pigment because this destroyed many of the landmarks both in respect to the regener-

ating tissue and the condition of the remaining old parts. Sections were sometimes depigmented on the slide. For this Mayer's chlorine method was used.

Table I gives in brief a record of the individuals operated upon, the character of the operation and the end results.

III THE NORMAL ADULT EYE

Before taking up the discussion of the regeneration of the eye it will be perhaps well to give a brief description of the structure of



Text Fig. 1 The lines, *a-b*, *a-c*, *a-d*, *a-e*, *a-f*, *a-g* and *a-h* represent approximately the different levels at which a part or whole of the eye has been removed. When the cut was at level as low as *a-e* a part of the optic ganglion was usually involved. In *Palæmonetes* the eye never regenerated when the cut came as low as the level *a-e*. Hermit crabs may regenerate an eye from the level *a-f* and a heteromorphic appendage from the level *a-h*.

the normal eye. The nature and extent of the operations and the subsequent changes in the eyes will then be more easily understood. The eye of *Palæmonetes* has been concisely described by Parker ('97) and the following description is adapted from his. It is to be noted that the eye structure of the three forms experimented upon is practically the same.

TABLE I.
PALAEMONETES

Series	No. of individuals	Operation	Date		Died from operation	Lived and moulted	Showed regeneration
I	16	part of cornea removed	May	10	12	4	0
II	12	part of cornea removed	July	11	8	2	1
III	38	part of cornea removed	July	20	29	9	8
IV	40	part of cornea removed	July	28	28	11	8
V	15	part of cornea removed	July	30	3	12	0
VI	16	part of cornea removed	August	1	12	2	1
VII	15	part of cornea removed	August	7	10	3	3
VIII	12	part of cornea removed	November	5	5	5	0
IX	27	part of cornea removed	November	9	8	9	0
I	15	entire cornea removed	March	10	4	7	0
II	18	entire cornea removed	July	20	8	9	1
IV	30	entire cornea removed	July	29	28	2	0
V	30	entire cornea removed	July	30	20	9	0
VI	13	entire cornea removed	August	4	8	5	0
VII	12	entire cornea removed	December	30	11	1	0
		entire eye removed					
I	12	both eyes	January	1	4	6	0
II	12	entire eye	March	5	9	3	0
III	12	both eyes	March	5	12	0	0
IV	18	both eyes	March	10	10	5	0
V	45	entire eye	April	19	12	20	0
I	20	eye split	March	5	5	12	3
I	28	eye split	May	24	16	12	0

HERMIT CRAES

I	12	cornea removed	March	25	2	8	3
II	12	cornea removed	May	26	2	7	2
III	25	cornea removed	July	9	2	16	5
I	15	entire eye removed	May	26	1	12	1
II	25	entire eye removed	July	9	4	14	9
III	8	entire eye removed	October	16	0	3	0
IV	12	entire eye removed	November	27	2	6	0

CRANGON

I	20	part of cornea removed	August	4	0	19	10
I	22	eye removed	August	4	1	19	1

NOTE.—Removing the entire corneal portion of any of the forms was always accompanied by the removal of part of the optic ganglion. In many cases as much as half of it. It frequently resulted that all of the corneal portion subsequently degenerated after a part of it had been removed, and it also frequently happened that the removal of the entire corneal portion resulted in the loss of the whole eye stalk, consequently the above table can be used only as a very general indication of the character of the operation.

The compound eye may be regarded as that part of the optic apparatus contained in the eye stalk. It consists of a large number of ommatidia occupying the distal end of the stalk and a series of four ganglia which extend through the axial portion of the stalk. This series of ganglia for present purposes may be regarded as a compound ganglion composed of four rather distinct sections or ganglionic masses united to each other by nerve fibers. The ommatidia are connected with the distal section of the optic ganglion by the retinular nerve fibers. The optic nerve passes inward from the proximal section of the ganglion to unite the eye with the brain. The basement membrane forms a sort of partition between the ommatidia and the optic ganglion. The transparent chitinous covering over the ommatidial region is known as the cornea.

Each ommatidium is composed of the following cells: two corneal hypodermal cells, four cone cells, two distal retinular cells, eight proximal retinular cells one of which is rudimentary, and a variable but small number of accessory pigment cells. Black pigment granules are contained in both proximal and distal retinulæ and are found only in these cells. The yellowish pigment is confined exclusively to the accessory pigment cells. The different cells enumerated above give rise to the structures that constitute a complete ommatidium. The two corneal hypodermal cells secrete the square corneal facet which covers the outer surface of the ommatidium. Immediately beneath the corneal hypodermal cells is the crystalline cone formed by the four cone cells. The nuclei of these cells are located in their distal ends. The main body of the cone appears as a dense hyaline secretion. Proximally the cone is less dense in structure and tapers to a slender stalk lying between the cone and rhabdom. The rhabdom, according to my observations, is a swollen spindle-shaped structure proximal to the inner ends of the cone cells. The distal retinular cells lie near the inner end of the cone; the proximal retinulæ surround the distal end of the rhabdom. The proximal processes of the retinulæ extend over the rhabdom and pass through the basement membrane as the retinular nerve fibers to enter the optic ganglion below. The accessory pigment cells lie both above and below the basement membrane.

Before closing the description of the normal eye mention should be made of another point. In young individuals in each of the species examined in this series of experiments there is present a growth zone in the ommatidial region. From this zone the number of ommatidia is increased as the animal grows older and increases in size. In longitudinal sections cut in a horizontal plane this zone is apparent on the inner edge of the eye as a narrow band of elongated cells situated above the basement membrane and between it and the completely developed ommatidia. In some instances partially differentiated ommatidia can be recognized in this growth zone. This zone has been mentioned by Parker and others. It is briefly described in this connection now because it is probable that in some instances ommatidia that have apparently regenerated have in reality developed from the growth zone.

The terminology used in the above description and in the discussion of the regenerating eye is that used by Parker. The series of optic ganglia described by him as occupying the eye stalk, however, have been referred to in this paper as different divisions of a single ganglion, it being thought that the matter could be treated with less confusion in this way. Also, the distal portion of the eye stalk, called the retina by Parker, is referred to here as the ommatidial portion. It is composed of a large number of individual ommatidia and the more general use of the term, retina, does not imply all the structures composing the ommatidia. The structure of the rhabdom as described in the present paper is not in full accord with Parker's description.

While the above description applies especially to *Palæmonetes* it is sufficiently accurate for the other forms described in this work to serve all purposes. The most marked differences in the structures of the eye of *Palæmonetes* and of the other forms used are as follows: In *Crangon* and in crayfish the eye stalk is much shorter and proportionately greater in diameter than in *Palæmonetes*. Also, in *Crangon* some small glands are found located just below the basement membrane. Hermit crabs have long slender eye stalks similar to those of *Palæmonetes* except that at the base and occupying the dorsal inner edge there is a small pointed squame

bearing a number of sensory hairs. (Fig. 37, *o.sq.*) And finally, there are no accessory pigment cells in the hermit crab's eye. These cells are very conspicuous in the eyes of *Palæmonetes*.

IV THE PRELIMINARY REGENERATIVE PROCESS

The preparatory stages leading to the regeneration of an eye stump consist chiefly of the following processes; the healing of the wound, the removal of the injured tissues and the active proliferation of new cells of a comparatively undifferentiated character. In removing any part of the eye the injury to the remaining soft, inner tissues is considerable. Especially is this true when the cut passes through the ommatidial region. Much of the tissue surrounding the wound is crushed and torn out of place. On this account the process of healing over the cut surface is much more difficult to follow than the healing of the wound after an antenna or leg has been removed.

Before taking up the description of the preliminary regenerative process it will be perhaps of interest to give in brief the immediate effects of the operation. The death of the animal which so frequently follows close upon the operation seems often to be due chiefly to nervous shock. It cannot be caused by loss of blood alone for usually there is no profuse bleeding. When the eye of *Palæmonetes* is operated upon the animal often turns over and over ten to forty times as soon as it is released and returned to the water. Many of the animals die before they succeed in righting themselves. Others lie upon their sides several hours after they have ceased revolving and die without showing any normal activities or regaining their equilibrium. It very seldom happens that animals which whirl over and over many times after the operation ever recover from its immediate effects. These apparently helpless motions indicate that the operation has caused the loss of equilibrium.

Crayfish sometimes exhibit these uncontrolled whirling movements. Similar movements are also noted in fresh-water *Gammarus* and in *Ascellus*. In *Gammarus* and *Ascellus* these movements are executed after the removal of the antennæ or some of

the mouth parts. This shows that the effects are not specifically connected with operations upon the eye. Whatever its initial cause the effect is transmitted to the whole nervous system.

In many cases the operation seemed to affect the animal more seriously when only the upper part of the eye was removed than when the entire eye was cut off. Frequently the relative number of survivors was greater in the latter than in the former case. In other instances the animal did not seem greatly affected by the operation regardless of whether the whole or a part of the eye was removed. (See Table I.)

The immediate visible effects upon the eye may be briefly described as follows. As soon as any part of the corneal covering is removed or even as soon as a rent is made in it a considerable amount of the soft, viscous, inner tissue flows out through the opening. It is perhaps carried out by the escaping blood. Much of the pigmented, reticular tissue seems to escape, perhaps because it is softer and more viscous than the other tissues. After an hour or so the remaining, inner tissues are seen bulging out and above the general level of the surface. This is probably on account of the destruction of the normal tension of the tissues due to the changed pressure conditions at the wounded surface. A similar appearance is obtained when the surface injury consists of a rent torn in the cornea with a needle.

A HEALING OF THE WOUND AND FORMATION OF NEW CUTICLE

In a few hours after the operation most of the pigmented tissues have disappeared from the surface of the wound and the swollen surface takes on a whitish appearance. This white swollen surface is apparent for several days. Not until the fourth or fifth day is there any sign of the characteristic red-brown crust which generally forms over wounds in Crustacea.

Sections of an eye fixed six and a half hours after the operation show no definite indications of the healing of the wound. A great deal of the broken and mangled tissue lies outside the wound and hanging to the cuticle about its edges. Inside, the tissues are twisted and misshapen. At the edges of the wound there are

slight indications that it is preparing to close over. But the quantity of material lying inside and out makes it impossible to determine what tissues are taking part in closing the wound.

During the next twelve or fifteen hours the changes are still not clearly defined. The interior still presents a rather badly confused mass of injured tissue. Near the edges of the wound, however, there are evidences that the hypodermis has begun to push outward to cover the cut surface. For the most part the wound shows a smooth even surface which indicates that the passage outward of the injured tissues has ceased and that a sort of equilibrium had been established. The mass of tissue closing the cut seems to be made up of a few hypodermal cells and cytoplasmic strands, a considerable accumulation of blood cells and the nuclei of the breaking down tissues of the eye. Around the edges of the cut occasional strands of hypodermis with a very few nuclei can be distinguished.

Sections of an eye fixed about forty hours after the operation show the beginning of crust formation. Almost the whole surface has been covered. Judging by the reaction to stains, the part which may be considered the matrix of the crust is formed by an attenuated, chitinous secretion of the hypodermis. In this matrix are embedded numerous nuclei of the injured tissues together with a great many blood cells and a few hypodermal cells. In some parts the crust is sharply marked off from the underlying tissues by a space filled with coagulated plasma. Over one part of the wound the crust is not yet fully formed. At this point hypodermal strands containing elongated flattened nuclei are seen stretched across the space still uncovered. The strands appear in two or three layers with very few nuclei in each layer. No definite centers of cell proliferation can be recognized at this time.

After the crust has covered the cut surface it continues to increase in thickness for two or three days, then hardens, turns a bright reddish brown color and remains over the stump until a moult has occurred. The crust takes no further active part in the healing and regenerative processes. Fig. 46 represents in a semidiagrammatic manner the crust formed over the wound in a crayfish eye about sixty hours after the operation. The crust is continu-

ous with the inner surface of the cuticle covering the rest of the eye. There are still masses of the injured inner tissues that have been excluded by the formation of the crust clinging to its outer surface. The old tissues in the interior of the eye stump have shrunk back from the crust leaving a considerable space occupied by coagulated plasma. The old cuticle and the matrix of the crust both stain deeply either with orange G or acid fuchsin.

No distinct cuticle can be recognized for several days, from six to eight, after the operation. In the eyes of *Palaemonetes* that have moulted seven or eight days after the infliction of the injury a cuticle which corresponds approximately in thickness with the cuticle covering the remainder of the eye has formed over the wound. This new cuticle is much looser in texture than the old cuticle. Regeneration does not take place so rapidly in crayfish as in the marine forms examined so that a new cuticle is somewhat longer in forming. Frequently a considerable space intervenes between the overlying crust and the cuticle which has formed beneath it. This is probably due to the recovery of the tissues of the stump from their early swollen condition during which time they were gorged with blood and occupied more than their normal amount of space. It is not unusual to find considerable spaces between different layers of the new cuticle as if a shrinking of the tissues had taken place during the process of forming the new layers of cuticle. The shrinking of the interior tissues without doubt also accounts in part for the folds and wrinkles which often appear in the cuticle over the wound.

The secretion of the new cuticle which grows over the wounded surface begins some little distance back from the cut edges of the old cuticle and is continuous with its inner layers. Fig. 47 is a semidiagrammatic representation of the relation of the old and the new cuticle and the exclusion of the broken down tissue by the development of the new cuticle beneath it. Only eight nuclei appeared beneath the new cuticle in the section as shown in Fig. 47. This figure is taken from an eye that had been injured by tearing through the cuticle with a needle. Reference to the figure shows that very little of the old cuticle had been removed. Fig. 50 shows part of a section near the edge of the wound, Fig. 51 part of a

section near the center of the wound. Both figures show the distribution of the hypodermal nuclei beneath the new cuticle that had formed over the cut surface of the eye of a *Palæmonetes* ten days after the operation. Examination of Figs. 47, 51 will show that a new cuticle may be secreted before a complete hypodermis can be recognized.

Recently hatched *Cambarus gracilis*, 12 to 15 mm. in length had eyes injured by tearing through the cornea with a sterilized needle. The eyes operated upon in this manner were fixed at different times, varying from eleven to thirty-five days. All of the eyes, however, were fixed before a moult had taken place. In this way it was possible to determine the precise position of the original injury. Sections of such eyes show one point conclusively, at least for *Cambarus gracilis*. That is that the proliferation of new cells begins from the hypodermis immediately surrounding the rent. From the edges of this proliferating center new cells push out to replace the cells that were removed or that have broken down. Previous observations made upon the regeneration of the eye in crayfish (Steele '04) indicate that crayfish probably do not regenerate a functional eye. It appears, however, that the preliminary regenerative processes are essentially the same in crayfish as in the other forms examined.

It is frequently the case that a much greater proportion of the soft inner tissues are destroyed than of the outer cornea or the hypodermal layer beneath it. When the cut is made the retinulae and the lower ends of the cone cells press out of the wound and leave the outer ends of the cones and the hypodermis practically undisturbed. Such a condition is particularly noticeable in eyes that were operated upon by tearing the cornea with a needle. In such cases the hypodermal cells *in situ* secrete the new cuticle. This cuticle is, however, without corneal facets, a fact which shows that while the operation neither removed nor caused the disintegration of the hypodermal cells it still affected their activity to such an extent that they no longer function in their usual specialized manner. They now function as the ordinary hypodermal cells over the general surface of the body.

Sections frequently show a morphological transformation of the

corneal hypodermal cells *in situ* that are engaged in the secretion of the new cuticle. In the normal adult condition the pair of corneal hypodermal cells that belong to each ommatidium appears as much flattened cells crowded between the distal ends of the cones and the corneal facets. Their nuclei stain faintly and appear to be slender oval bodies lying flat against the cuticle. As the distal ends of the cones in an injured eye break down the nuclei of the corneal hypodermal cells enlarge, become rounded, stain deeply and in every way show signs of increased activity. Fig. 52 includes a series of figures showing the transformation of the corneal hypodermal nuclei into the larger, more deeply staining type seen in the regenerating eye. *a* and *b* in this figure represent the corneal hypodermal cells as they appear in the normal ommatidia. The other figures of this series, *c*, *c'*, *d* and *e*, show corneal hypodermal cells, belonging to ommatidia that have degenerated either wholly or partially. In *c*, *c'*, *c* the distal ends of the cones still remain almost intact and in *c* the nuclei of the corneal hypodermal cells appear but little larger than those associated with normal ommatidia. In *c'* and *e*, however, the nuclei of the corneal hypodermal cells are much enlarged, stain deeply and the cytoplasm surrounding them appears granular and loosely reticular. One nucleus to the left of the figure in *d* appears to be preparing to divide amitotically. That the nuclei shown in *d* are the transformed nuclei of the original corneal hypodermal cells is determined by the fact that on either side of these nuclei are others still associated with partially disintegrated cones. Their original character is also suggested by the fact that they are grouped in pairs. A regenerated, rather than a transformed, hypodermis over the ommatidial region never shows the nuclei arranged in pairs in the early regenerative stages. That the new cuticle has been secreted by these transformed hypodermal cells is shown by the relations of the two structures. The cytoplasmic strands of the hypodermis are continuous with the inner layers of the cuticle (Fig. 52*d*).

Of course it is not absolutely proved that the transformed hypodermal cells take part in the later regenerative processes. This could not be done without examining a very great number of

stages. But even the examination of a series of sections from the same eye will show that the indications are strongly in favor of the view that these hypodermal cells remain active and constitute the hypodermis during the subsequent regenerative processes. Fig. 53 is taken from the same eye as *d* in the series shown in Fig. 52. In the later figure it is evident that regeneration is in progress. The hypodermal cells, however, show a tendency toward a paired arrangement indicating that they are the original hypodermal cells.

After a part of the corneal covering has been removed it is evident that an entirely new hypodermis must be regenerated over the wounded surface. In crayfish it was seen that active cell proliferation began near the edges of injured hypodermis and that new cells pushed outward from these centers. The early stages have been examined in a number of eyes of *Palæmonetes*. The centers of cell proliferation in this form are not so apparent. The nuclei which in the early stages appear beneath the cuticle that covers the wound are very few and lie far apart. Their number increases not chiefly by the repeated multiplication of nuclei at the edges of the wound but by the repeated division of the nuclei that are pushed out onto the wounded surface. During these early stages while the nuclei are actively dividing the cytoplasm is very loose and reticular and the cell boundaries are indistinguishable.

In many cases the new cuticle which becomes apparent after the first moult is somewhat definitely separated into a dense outer portion and a semifibrous inner division. The inner division often appears as an interlacing network of fine fibers, many of which can be traced into the hypodermis beneath (Fig. 53). The loose incompact character of the hypodermis over the wounded surface probably indicates a high degree of activity of the hypodermis in this region.

It is apparent from the foregoing description of the formation of the hypodermis covering the wound that the regenerated hypodermal cells may arise in two ways. They may arise by a transformation of the old corneal hypodermal cells *in situ* in which they assume a less specialized rôle or they may arise by the migration of a limited number of hypodermal cells from the edges of the

cut, which later multiply until a complete hypodermis is formed. In either case the first new nuclei must be contributed by the remaining hypodermal cells. Whenever the cut has not removed the entire ommatidial portion the remaining corneal hypodermal cells must assume a somewhat less specialized rôle in order to form the first new nuclei of the regenerated hypodermis.

B REMOVAL OF THE INJURED TISSUE

The fact that the inner tissues of the eye are so much softer than the chitinous outer covering renders it impossible to operate upon the eye in any way or to remove any part of it without serious injury to the remaining softer tissues. It is evident that, before any considerable regeneration can take place, the injured tissues must be either repaired or removed. All the observations made upon regenerating eyes tends to show that none of the injured tissues except the hypodermis ever repair themselves.

Sections of an eye fixed six and one-half hours after the operation show that considerable changes have already taken place in the injured tissues. The effect is particularly noticeable in the retinulæ. Many of the reticular nuclei have become separated from the pigmented reticular processes and appear as rounded bodies surrounded by a dense mass of cytoplasm. These are irregularly scattered among the other tissues. Some parts of the interior have become fairly clear of the broken down structures and are occupied chiefly by coagulated plasma. In other parts of the eye the injured tissues lie in confused masses.

The changes in the next twelve or fifteen hours do not show much advance over the earlier stages. The interior still presents a badly confused mass of broken down tissue. In some parts, however, the cone nuclei appear larger, the bodies of the cones have begun to dissolve and the number of rounded reticular cells appear somewhat more numerous than in the earlier stages, their nuclei showing irregularities in shape (Fig. 75*a* and *b*).

During the earliest stages, six to sixty hours after the operation, the ommatidia that are still intact always appear bent and twisted out of their normal positions. Later stages show that these

uninjured ommatidia have regained their original shape and position. This temporary contortion of the ommatidia seems to be due to the immediate effects of the operation, reduction of pressure, destruction of normal tension relations, etc. That in the later stages they appear normal again indicates that they have adjusted themselves to the new conditions imposed upon them by the operation.

Besides those ommatidia that are actually injured by the operation a large portion of those remaining frequently degenerate. The destruction of the tissues of the eye is, consequently, much more extensive than the original injury. This fact is strikingly illustrated by eyes in which the original injury consisted in thrusting a needle into the ommatidial portion. In several such instances the entire, or almost the entire, eye has degenerated. Instances of this kind have been observed in the eyes of several *Palæmonetes* and also in the eyes of young *Cambarus gracilis*. Similar phenomena have been observed in eyes of fresh water *Gammarus*. To be sure the eyes of *Gammarus* are quite small which may account in a measure for the fact that in six or eight eyes examined in serial sections only one showed any of the old ommatidia intact. In *Cambarus gracilis* there were two instances out of six in which none of the ommatidial portion remained. In one of the cases the entire eye had degenerated; not even the vestige of the stalk remained. All of this degeneration took place in about thirty days and without a moult. In the other case all of the ommatidial portion and more than half of the optic ganglion had degenerated during the same period. In other eyes of the same series very little degeneration followed the operation. In these extreme cases it seems probable that some infection played a part.

In the degeneration and removal of the injured tissues the retinulæ degenerate most rapidly. In many cases they break down within the first few hours after the operation. Their long pigmented processes become separated from the cell body and collapse into shapeless masses of pigment, which become scattered through the other tissues. The greater part of this pigment finally gathers in clumps near the level of the basement mem-

brane. Although the retinulæ are the first to collapse their remains are the last to be gotten rid of. Evidently the pigment is absorbed and removed only with difficulty. Often in regenerating eyes that have completely regenerated new ommatidia much of the old pigment remains.

The cell bodies of the retinulæ after losing their pigmented processes appear as large nuclei surrounded by a narrow zone of condensed cytoplasm. Within a short time these cells become scattered widely through the eye. After a few days their nuclei appear irregularly shaped and soon afterward become conspicuously polymorphic (Fig. 75*a* and *b*). In the usual course of events these retinular cells disintegrate and disappear. But under some conditions they apparently remain and later multiply and give rise to an abnormal development of tissue that secretes pigment.

The rhabdoms and the inner ends of the cones also degenerate within a short time after the retinulæ. The cones continue to dissolve from the proximal ends distally. The last parts to disappear are the outer ends in which are embedded the cone nuclei. Before the dissolution of the cones is complete the cone nuclei appear greatly enlarged and stain deeply. Their enlarged appearance is probably largely due to the disintegration of the cone substance from about them.

As the disintegration of the tissues proceeds the interior of the eye becomes filled with a granular mass containing scattered nuclei and masses of pigment. This granular mass which is usually very much vacuolated is made up of the remains of the old tissues together with more or less of coagulated plasma and blood cells. Sometimes the remains of the old cones appear as long tapering bands of granular material extending from the periphery inward.

Fig. 51 represents a small area of the disintegrated ommatidial structures as it appeared in the eye of *Palæmonetes* ten days after the operation. Only two or three nuclei lie close beneath the cuticle and a few others lie scattered deeper in. Most if not all of these more deeply located nuclei are the remains of the old ommatidial structures. In the lower part of the figure are some old

pigment remains. The granular mass occupying the greater part of the figure is made up chiefly of partially dissolved cones. The slender strands which can be traced through the granular mass are in part at least made up of new cytoplasm. At certain points the cytoplasmic strands are seen to be continuous with the inner layers of the cuticle.

Fig. 48 is taken from a section of an eye of *Palæmonetes* seven days after the removal of part of the ommatidia. A surface view from which this figure is taken is shown in Fig. 1. Practically all the material represented in Fig. 48 except the cuticle is made up of disintegrated ommatidia. The long band extending inward shows approximately the position of a former cone. The large vacuolate spaces in the upper part of the figure are old nuclear remains. In the process of disintegration the nuclei at first enlarge and stain deeply. Later the nuclear contents disappear although the nuclear membrane persists for some time longer, often becoming shrunken and folded into a variety of shapes.

It has been seen that the disintegration of the injured tissues begins immediately after the operation and that the greater part is accomplished in from one to three days. The distal ends of the cones alone remain intact for a much longer time, in some cases from two to three weeks and occasionally even longer. The removal of these disintegrated tissues is much slower than their dissolution.

Regeneration proper may and usually does begin within a few days after the old structures have broken down and progresses simultaneously with their removal (Figs. 49, 50). One part of the eye may show ommatidia differentiating while another region is still occupied by disintegrated old structures. The individual differences in the rate of regeneration of such frequent occurrence is probably largely dependent upon the variations in the length of time required for the removal of the old structures. This probably also accounts for the fact that regeneration does not take place uniformly throughout the same eye. The part of the eye that gets rid of the injured tissue soonest regenerates first.

The above observations apply in general to all the forms used. Crangon, however, offers a significant exception in that the injured

tissues disintegrate much more slowly than in *Palæmonetes*, hermit crabs or crayfish.

C METHOD OF CELL DIVISION

In the earlier preliminary stages of the regenerative processes it is impossible to distinguish cell outlines. We should therefore speak of nuclear divisions, perhaps, instead of cell divisions. In later stages the cytoplasm becomes differentiated about the individual nuclei. In all cases of the regeneration of the eye the nuclei are increased by amitotic division. Before a definite hypodermis is established the nuclei can be seen in various stages of constriction, separating off new nuclei for the development of the future underlying structures. There seems to be no perfectly definite manner in which the constriction and separation of a nucleus into two parts takes place. One or two characteristic forms, however, appear so frequently as to be readily distinguished. The two most usual types are seen in the three nuclei occupying the extreme right of Fig. 53. When a nucleus divides in a plane parallel to its long axis it usually assumes the form of the outer one of the three referred to. The formation of the notch on one side gives the nucleus a peculiar heart-shaped appearance which seems characteristic and is easily recognized. Figs. 49 and 53 show nuclei of this same type further advanced in division. The other type referred to is represented by the other two nuclei of the three at the extreme right of Fig. 53. Apparently these two nuclei were recently formed by the longitudinal division of one of the heart-shaped nuclei, like the one lying beside them. Each of these two is now dividing unequally by a transverse constriction.

A dividing nucleolus can sometimes be seen but more frequently a definite nucleolus cannot be distinguished. When nucleoli are seen in dividing nuclei they usually appear with a darkly staining strand of material connecting them. In sections of young *Cambarus gracilis* eye, nine and sixteen days after the operation, two nucleoli can be recognized in many of the nuclei. In those nuclei that are dividing one nucleolus lies in each part. In all except in the later stages of regeneration of the eye nuclei dividing amitot-

ically can be found in great abundance. But at no time are mitotic divisions seen. In a careful examination of a large number of sections only one cell has been found that suggested the possibility of its being in mitotic division. The appearance of this one suggests a late anaphase or an early telephase. Consequently it is not certain that this is mitotic division. This nucleus appears near the left edge of Fig. 53 at *K*. In any case it must be admitted that amitosis is the regular method of cell division in regenerating eyes of the forms studied, since in every specimen examined during the stages of cell divisions amitosis has been observed and mitosis has not been seen.

That such is the case is somewhat unexpected since Miss Reed ('04) found mitotic division abundant in the regenerating leg of a crayfish. Miss Reed, however, observed that there were no mitotic figures during the early stages of regeneration, although new cells were being rapidly formed. Perhaps we may infer from this that amitosis took place in the regenerating leg of the crayfish during the preparatory stages at least. But one would hardly expect such differences in cell division in forms so closely related for example as crayfish and *Palæmonetes*. No observations have been made upon histogenesis in the regenerating appendages of hermit crabs, *Palæmonetes* or *Crangon*. Hence it cannot be said whether or not the eye furnishes an unique exception to the regeneration of other parts in these forms.

Recently, however, many have come to regard amitosis as a phenomenon of more frequent occurrence than has been generally supposed. Meves ('91 and '94), McGregor ('99), Child ('04 and '07), all describe amitosis as a normal phenomenon and consequently no longer accept Vom Rath's view that a cell is nearing its final dissolution when it begins to divide amitotically.

On the other hand many of the more conservative investigators are unwilling to admit that amitosis occurs as a normal phenomenon and believe that the apparent cases of amitosis can be explained on some other grounds. But in all the instances hitherto described amitosis has been found occurring along with mitosis. In the present case, however, all of the cell divisions are amitotic and they all take place in cells derived from the hypodermis; in

these respects the regenerating eyes investigated in this series of experiments offer a unique instance in their method of cell division.

V REGENERATION OCCURRING AFTER DESTRUCTION OF DISTAL PART OF EYE

Under this heading will be discussed the results obtained from eyes injured in varying degrees but not exceeding the destruction of more than the distal two sections of the optic ganglion. The injury originally inflicted varied from tearing the surface of the cornea with a needle to cutting off the whole top of the eye so that at least the first part of the second section of the optic ganglion had been removed. Frequently, however, as has been explained in Section IV the part of the eye ultimately lost was much greater than that originally removed. For in cutting the eye with scissors or tearing it with a needle much of the tissue surrounding and underlying the wound was so injured that it afterward degenerated.

In the series of experiments to be described under this division nearly two hundred *Palæmonetes*, twenty *Crangon* and fifty hermit crabs were used. Fifty per cent of the *Palæmonetes*, 5 per cent of the *Crangon* and 10 per cent of the hermit crabs died of the operation. Of those that survived the loss of blood and the nervous shock of the operation a considerable number died from other causes without having undergone a moult. Forty-five *Palæmonetes*, seventeen *Crangon* and twenty-nine hermit crabs, however, lived through at least one moult. Among these a number showed no regeneration either from surface examinations or from sections. The greater number of those that gave surface indications of regeneration and for comparison a number that showed no regeneration have been sectioned and examined.

A REGENERATION OF THE FUNCTIONAL EYE

Experience has shown that the number of days an experiment covered serves to indicate only in the most general way the stage of regeneration. While the time element naturally constitutes a most important factor the rate of regeneration is also dependent upon the season of the year, the age and the physiological activity

of the individual and perhaps other factors not so apparent. Besides individual differences displayed between members of the same species there also appeared to be differences in the ability to regenerate and the rate of regeneration among the three species chiefly used, these differences being much more marked in the regeneration of an eye than in the regeneration of an appendage. The hermit crabs seem to completely regenerate an eye in shorter time than either *Palæmonetes* or *Cragon*. But the final results were similar in all three forms. The few significant differences will be pointed out and discussed later.

1 Entire Preparations of Regenerating Eyes

A careful examination of the regenerating eye frequently reveals a number of important features and is absolutely essential to the later interpretation of the sections. Therefore outline surface drawings have been made of all eyes later sectioned. In most cases the normal eye was drawn in connection with the regenerating eye for comparison as to size, shape, etc. Frequently the two eyes were sectioned together. These surface views which had better be regarded as optical sections were drawn with a camera after the eyes had been brought into oil preparatory to embedding. Figs. 1 to 25 represent various stages of regenerating eyes.

Fig. 1 shows dorsal and ventral views of a *Palæmonetes*' eye seven days after an operation which removed the ventral corneal surface and immediately after a moult. A comparison of the dorsal and ventral views shows that the injury is confined chiefly to the underside. In shape the eye is practically normal. It is not even flattened on the underside as the thinning out of the pigment near the center would seem to indicate. As is usually the case the injured eye is much smaller than the uninjured one. Not only the region operated upon but the whole eye decreases in size after the operation. This indicates that however localized the operation may be the effect is much more extended. In this particular case the part of the eye proximal to the injury measures only three-fourths of the length of the same region in the uninjured eye.

Fig. 3 represents the ventral view of a *Palæmonetes* eye ten days after being injured. In this case also the injury extends across the ventral side of the ommatidial portion. The pigment of the broken down ommatidia can be seen scattered in flakes and patches through the upper part of the eye. From the dorsal side the eye appeared nearly normal but sections show that almost the entire eye is in process of degeneration.

The specimen represented in Fig. 4 shows a regenerating eye nineteen days after the removal of almost all of the ommatidial portion. It is readily seen that very little remains except the eye stalk. The pigment patches are remains of the original eye. Across the end of the stump the cuticle is wrinkled and folded, indicating that comparatively little new tissue has been formed and that the cuticle follows more or less closely the rough uneven outlines of the wounded surface.

When the entire ommatidial portion has been removed or has degenerated regeneration seems to be considerably slower than when a large part of it remains uninjured. The two specimens shown in Figs. 9 and 10 afford a striking illustration of this fact. Both of these eyes were operated upon at the same time. Each animal moulted twice, the first time on the same day and the second, a day apart. Both were fixed in picro-acetic at the same time, thirty-two days after the operation. In Fig. 9 the injury involved only the posterior ventral side, less than one-half of the ommatidia. While in Fig. 10 the injury included all of the structures lying distal to the basement membrane. Examination of the sections shows new ommatidia completely differentiated in Fig. 9 while in no part of Fig. 10 are they yet defined.

The next specimen, Fig. 20, presents a rather striking appearance and suggests immediately that the regeneration taking place is not altogether of the normal type. This is a thirty-eight day specimen and belongs to the same series of experiments as the preceding two specimens. All of the part distal to the dark pigmented band is regenerated tissue. The pigment consists chiefly of the remains of the old retinulæ. It is evident even from a surface view that no ommatidia have developed. Sections show, however, that on one side the differentiation of cones is beginning (Fig. 69). Here it

may be well to mention a fact that has been observed a number of times. The regeneration of the new ommatidia never presents a uniform stage of differentiation in any case whether or not all of the old ommatidia have disappeared. In fact it may be possible to select several stages from the same eye and sometimes two or three stages from the same section.

An eye thirty days after the injury, Fig. 6, shows some interesting features in comparison with the one just described. From a ventral view this eye shows no signs of remaining ommatidia and from the dorsal side only a very few cones and facets are evident. The upper part of the eye is transparent. Below this transparent area are scattered patches of pigment representing the remains of the old eye. Sections show that no new ommatidia have been entirely differentiated, that the retinular cells have differentiated and are establishing connections with the optic ganglion, that new cone nuclei are being separated from the hypodermal nuclei, that on one side a few of the old ommatidia remain and that in the tissues lying nearest the old ommatidia new cones are being developed. The most striking feature presented by this specimen is the clearness with which the connections between the retinulæ and the ganglion cells can be made out. These connections will be discussed at length in the consideration of the detailed structures of the regenerating eye.

Fig. 17 shows the ventral view of a regenerating *Palæmonetes* eye from a thirty-five day specimen. Examination of the dorsal side shows that a large number of the ommatidia on that side appear uninjured, the greater part of the injury being confined to the ventral side as indicated by a surface examination. Sections show, however, a gradation of regeneration from a stage in which there is no signs of cone differentiation to the complete formation of a new ommatidium.

It was said above that hermit crabs regenerated an eye more rapidly than either *Crangon* or *Palæmonetes* even in instances where a considerable portion of the optic ganglia had been removed. Figs. 5, 12 to 15 show regenerated eyes of hermit crabs after one or more sections of the optic ganglion have been destroyed. Fig. 12 had at least the distal division of the ganglion

removed. The regenerated eye shown in the figure was developed within thirty-three days and after only one moult. This moult occurred on the thirty-second day and the eye was fixed in Perenyi the thirty-third day. Sections show that ommatidial structures have been fully differentiated although incompletely developed. One point is particularly noticeable in these sections; the ommatidia are very much shorter than in the normal eye. This condition was probably caused by the mechanical pressure of the covering cuticle which forced the developing ommatidia into less space than they would otherwise have occupied. Fig. 13 shows the eye of a hermit crab regenerated from a stump in which not more than half of the optic ganglion remains. The regeneration in this eye took place in thirty-eight days. One moult occurred twelve days after the operation. Although extremely small the eye is practically perfect except that the corneal facets have not yet developed.

Figs. 14 and 15 show two other regenerated eyes of hermit crabs forty-one and sixty-seven days respectively after the operation. Sections of the forty-one day eye do not show ommatidia as fully developed as the thirty-eight day specimen previously described. The sixty-seven day specimen shows the eye complete in all its details even to the corneal facets. Whether a younger regenerated eye might not show the corneal facets has not been determined since no specimens were available between the forty-one day and the sixty-seven day specimens.

A noticeable feature in all the regenerated eyes of the hermit crabs is their small size in comparison with the normal eyes. It is probable that the regenerated eyes would have increased in size if the experiment had covered a longer period of time. Sections of the eye shown in Fig. 12 indicate that the definitive size has not been reached. For, lying outside the fully formed ommatidia are others in the process of development. In the case of the eye shown in Fig. 13 sections do not show any indications of partially developed ommatidia and it may be that this eye would never have reached the size of the normal eye. Perhaps this is what we should expect since the amount of nervous tissue present is considerable less than is normal.

It is more difficult to interpret the actual condition of a regener-

ating eye in Crangon than in either *Palæmonetes* or hermit crabs. The whole of the eye stalk in Crangon is thickly covered with branching pigment cells. So that even after the eyes are brought into oil very little can be seen in detail by examining them in toto. Figs. 7 and 8 thirty-one and thirty-two days, respectively, are fairly characteristic of the regenerating eye of Crangon. From all that can be determined from the outside, regeneration seems practically complete in each of these cases. Sections show, however, that comparatively little regeneration has taken place. A fuller discussion of the regenerating eye of Crangon will be taken up elsewhere.

2 *Details of Development of the Regenerating Ommatidia*

The complete regeneration of the ommatidial portion of the eye involves three stages. These can be separated from each other rather sharply although they overlap more or less. The first stage consists in getting rid of the broken-down tissues and the healing of the wound; second, the active proliferation of new cells; and third, the differentiation of the new ommatidia. The first and second stages have been discussed in Section IV.

a Regeneration of Retinulae

All the observations support the conclusion that the regenerated ommatidia are derived entirely from the hypodermis. Before the hypodermis covering the end of the stump has been clearly differentiated, however, the proliferation of cells for the new structures lying below has begun. So that at the same time hypodermal cells are dividing in two planes, one at right angles to the periphery to increase the number of hypodermal cells, and the other parallel to the surface. The inner nuclei of the latter division migrate inward and become the first reticular cells. As they migrate they become elongated with their long axes radially arranged.

Fig. 54 shows the early stages of the separation of reticular nuclei, some separating from the nuclei at the periphery and others migrating in. At a comparatively early date these reticular nuclei have migrated a considerable distance below the surface

and may be seen in a relatively well defined row (Fig. 68). Soon after the reticular nuclei have been separated from the nuclei at the surface they themselves begin to divide (Fig. 68) in a plane at right angles to their plane of original division. These longitudinal divisions may begin before the nuclei have reached their definitive position. This division continues until a band composed of many nuclei has been formed. Figs. 60 and 62 show portions of such bands.

Here and there single nuclei are found lying much nearer the basement membrane than the reticular band (Figs. 60, 62). These are occasionally seen constricted. It has been impossible to determine with certainty the fate of these scattered nuclei but there are evidences which suggest that they become the nuclei of the accessory pigment cells.

The reticular nuclei even in the early stages of their migration stain much more deeply than the nuclei at the periphery. But at this stage no definite cytoplasmic outlines can be distinguished. Very faintly staining delicate strands of cytoplasm, however, can be found extending between the reticular nuclei and the periphery. These strands form an intermingled network and with the nuclei lie in a granular substance. Soon after the nuclei have reached their definitive position the cell bodies of the reticulæ can be recognized. Each nucleus appears surrounded by more or less definite strands of cytoplasm which are radially arranged and extend outward toward the periphery and inward to the basement membrane (Figs. 60, 65). These can now be definitely recognized as reticular cells. Usually it is easier to see the proximal than the distal strands for at an early stage these lower processes begin to secrete pigment and are consequently more conspicuous (Fig. 65). At the stage represented by Fig. 62 there is only the merest beginning of pigment deposition. The fibers are but little differentiated from their background which still seems to be composed largely of a homogeneous granular material, probably to a great extent coagulated plasma.

At a stage such as is represented by Figs. 60, 62, in which there is only the merest beginning of pigment secretion delicate cytoplasmic processes can be traced from the reticular nuclei inward

through the basement membrane beneath which they can be found branching over the ganglion cells. Fig. 62 shows several isolated reticular cells and their proximal processes which are seen extending through the basement membrane and reaching to the ganglion cells beneath. There is no evidence that these reticular fibers are directly connected with the ganglion cells; they seem merely to twine around them.

When the fibers have reached the basement membrane they may pass directly through it as the two shown in the left side of Fig. 62, or they may extend along the upper face of the basement membrane before entering the ganglionic mass below. The reticular processes frequently branch shortly before entering the basement membrane or just as they emerge below it. No special nerve methods were employed yet numbers of these fibers are seen branching among the ganglion cells and many are readily traced from the reticular nuclei to the basement membrane (Fig. 65). It is only in very favorable specimens that the fibers can be traced, however, through their whole length.

b Regeneration of the Crystalline Cones

Up to and including the stages shown in Fig. 65 and described above there is no evidence of any differentiation of the crystalline cones. A definite hypodermis, however, has been formed with an increased number of nuclei, from which other nuclei are separating. These are the cone nuclei (Fig. 63).

The formation of the cone nuclei does not appear to take place in a uniform manner. The usual method, however, is by the division of the hypodermal nuclei in a plane parallel to the periphery, the inner nuclei thus formed being the cone nuclei. But since in every ommatidium there are four cone nuclei and only two hypodermal nuclei, it is evident that either the hypodermal cells must divide twice or the first cone nuclei must themselves divide in order to make the cone nuclei just twice the number of the hypodermal nuclei. Observations indicate that in some cases the second pair of cone nuclei arise by the division of the first pair. In other cases, however, it is uncertain whether they arise in this manner or whether they arise from the hypodermal nuclei. It is

probable, however, that they are formed by the division of the first pair of cone nuclei as can be determined in some cases.

In the early stages of cone formation the inner surfaces of the hypodermal cells lose their distinctive outlines. For at this time there is no clear line of demarcation between the hypodermal and cone cells. Fig. 63 represents one of the earliest recognizable stages in crystalline cone formation. The hypodermal cells are more or less definitely grouped into pairs and it is readily seen that cone and hypodermal nuclei are not wholly separate. Extending inward from the cone nuclei are very delicate strands of cytoplasm. These strands seem to group the hypodermal nuclei into pairs and by their branching and crossing form a much vacuolated network.

At a stage slightly more advanced (Fig. 64) the cytoplasm of the cones has begun to assume a more definite cone shape. There is still, however, no distinct line of separation between the hypodermal and cone nuclei. Neither does the cone mass show the boundaries of its component cells. In stages a little later the cone cells begin to show individual outlines and the cytoplasm appears more condensed. Cone formation is practically complete, however, before the corneal hypodermal and cone cells show a distinct line of separation (Fig. 66).

As the cone cells differentiate the cytoplasm becomes less and less vacuolate and gradually assumes a dense granular appearance. The cytoplasm is most condensed just below the nuclei and decreases in density proximally.

In longitudinal sections of the cones the cell boundaries appear distinct from their outer ends inward to the outer retinulae. At this point the cell boundaries become indistinct and the cone tapers rather suddenly into a slender stalk which extends to the distal end of the rhabdom, where it ends abruptly (Fig. 66). At a somewhat more advanced stage the boundaries between the cone and corneal hypodermal cells become distinct, and the cone secretion takes on the dense homogeneous deeply staining appearance characteristic of that in mature cones (Fig. 67).

c Regeneration of the Rhabdoms

There is no indication that the retinular cells have begun to secrete the rhabdoms until after the cones have been distinctly outlined, although the retinulae themselves become clearly differentiated before there is any indication of the cones. Not until the differentiation of the ommatidia has reached a stage intermediate between these stages shown in Figs. 64 and 66 can the anlagen of the rhabdoms be recognized. The rhabdoms first appear as slender homogeneous rods. Each rod is of uniform diameter throughout its length, and is distinguishable from the inner ends of the cone cells only by the fact that it stains slightly deeper and shows no divisions which indicate that it is composed of more than one cell (Fig. 66.) The rhabdoms show no signs of the characteristic spindle-like form and the complicated system of transverse plates so noticeable in the normal adult eye until after the last stage in the differentiation of the cones (Fig. 67). Even at the stage shown in the preceding figure the rhabdom does not show a normal appearance of its spindle form and the pigmented extensions of the retinular do not cover it so completely as in normal adult ommatidia.

It is evident from the preceding description and accompanying figures that all the structures necessary to a completely regenerated eye have been laid down. It is also seen that with the exception of the corneal facets the regenerated ommatidia are practically identical with those of the normal adult eye. A specimen of later stage, however, shows both the corneal facets and the definitive form of the rhabdom, so that the regenerated ommatidia present a perfectly normal appearance even to the minutest detail (Fig. 76).

All observations show that the differentiation of corneal facets could not become evident until after at least two moults. The first cuticle which is developed is formed before a continuous hypodermis has grown over the wounded surface and before any regeneration of ommatidia has begun. Corneal hypodermal cells are not differentiated as such until all of the other ommatidial structures have been laid down. Therefore the secretion of corneal facets constitutes the final process in the regeneration of

ommatidia. Since this is true, several moults may occur before the corneal facets differentiate, and at least two must take place.

3 *Comparison of Ommatidia in Regeneration and Ontogeny*

In comparing the regenerating with the embryonic eye it is necessary to consider them only from the beginning of ommatidial differentiation, since there can be no exact parallel between the preliminary stages of regeneration and the mode of the origin of the embryonic eye. These two processes are similar, however, in the respect that the ommatidia in both cases develop from the hypodermal cells. All these observations upon the regenerating eye give evidence that the cells which take part in the formation of the new ommatidia are derived primarily from the hypodermal cells that cover the wounded surface. That the ommatidia of the embryonic eye develop entirely from the hypodermis is the conclusion of most observers. There is no further agreement, however, in details except in the case of embryonic eyes that are described as arising without invagination, *e. g.*, the compound eye of the honey bee as described by Phillips ('05) and of the lobster (Parker '90), in which it was found that the ommatidia are developed from a single epithelial layer and consequently from morphologically similar cells.

It has been seen that the first cells differentiated from the hypodermis in the regenerating eye are the retinular cells. This can be regarded as being in agreement with the conclusion of Phillips that the retinulæ constitute the morphological center of the ommatidium. At any rate the retinulæ are in each case differentiated before the cone cells can be recognized, none of the cells originally separated from the hypodermis to form the retinulæ ever take any part in the formation of cones, and finally the cone cells arise peripheral to the retinulæ.

The differentiation of the regenerating ommatidia, described in a preceding section, and of the embryonic eye, as described by Kingsley ('87), may perhaps be regarded as presenting a parallel. Kingsley finds the nuclei which go to make up the cones and retinulæ arranged in radial rows and that the outer and hence the

later formed nucleus of each row contributes to the formation of a cone while the remaining nuclei form the retinulæ.

Unlike the rows of nuclei described by Kingsley and the spindle shaped groups of cells described by Phillips the retinulæ of the regenerating eye do not appear to become separated into definite groups before the development of the cones. In sections from the same eye there may be groups of retinulæ somewhat distinctly separated from each other and other retinulæ which constitute a continuous band for a considerable distance. Figs. 60 and 61 illustrate these opposite cases. But even when groups can be recognized there is no certain indication that a group belongs to a single ommatidium. The group may contain a fewer or a greater number of cells than belong to a single ommatidium. Besides the retinulæ continue to divide occasionally up to the time the cones are differentiated. From the evidence furnished by a number of different specimens it appears that the definite separation of the retinulæ into groups does not take place until after the cones are well advanced in their development. As the cones differentiate from the periphery inward the retinulæ become grouped about them. As this grouping continues the retinular processes become more and more slender, perhaps largely as a result of mechanical pressure.

The development of the cone as shown by my observations is the result of intra-cellular secretion. In this respect it agrees with the embryonic development of the cones in the eye of the honey bee as described by Phillips. It is directly opposed to the method described by Patten ('87), Kingsley ('87) and Watase ('89), who regard the cones as the result of the extra-cellular secretion of the cone cells.

The evidence furnished by the regenerating eyes of *Palæmonetes*, Crangon and hermit crabs agrees with the observations of those who do not find the cone and the rhabdom to be developed in the embryonic eye as continuous structures. Some investigators regard the rhabdom as merely an inward prolongation of the cone cells. Kingsley finds such a relationship in the embryonic development of the eye of Crangon. Patten regards the cone as extending from the hypodermis to the basement membrane and as differen-

tiating at the lower end into the rhabdom in most cases. But in *Vespa* he describes the inward prolongation of the cone cells as enclosing the rhabdom. Parker ('00) describes the prolongation of the cone cells in *Homarus* as extending to the basement membrane and inclosing the rhabdom in the same manner. The description of the relation of the rhabdom to the cone in *Crangon*, given by Kingsley, and applied to Crustacea in general by Patten, does not agree with the facts presented by the regenerating eyes of *Crangon*, *Palæmonetes* and hermit crabs. Obviously, however, this interpretation is in accord with that of Phillips in the case of the honey bee and that of Grenacher ('74), both of whom find that the rhabdom is developed as a secretion of the retinulæ, and do not find the cone cells extending as slender processes beyond the distal end of the rhabdom.

Concerning the source and manner of the innervation of the ommatidia the results obtained in this study of the regenerated eye agree only with those observers who, like Parker ('91) and Phillips ('05), regard the reticular cells as hypodermal sense cells which send nerve fibers into the ganglion below. It is true that no special nerve methods were used in this work upon the regenerating eye. But in some specimens at least the prolongation of the reticular processes into fibers which penetrate the optic ganglion is clearly evident (Fig. 62). In many other cases the processes can be traced from the reticular nuclei to the basement membrane and similar processes are found branching among the ganglion cells below it. But in no case is there the slightest evidence that the ganglion cells are sending fibers upward to the regenerating ommatidia. Consequently there seems to be no room for reasonable doubt that the retinulæ form the nerve endings of the ommatidia.

In this particular these results differ from those of Patten, Kingsley and other workers on the embryological development of the eye of certain Decapods. These investigators regard the nerve connections as being formed by the extension of processes upward from the ganglion cells, through the rhabdom and into the cone.

The observations made in this work seem neither to uphold nor to oppose the views of those who find that the ganglion cells send

processes upward into the retinulæ during the embryonic development. For it is not inconceivable that the innervation of the ommatidia of a normal eye should be accomplished by the upward growth of processes from the ganglion cells to the retinulæ and that in the regenerating eye it should be accomplished by processes growing inward from the retinulæ to the ganglion cells. That this is not impossible is suggested by the fact that in regeneration tissues are sometimes developed from the same germ layer while they arise from different germ layers in embryonic development.

Several instances are known where muscles in regenerated appendages arise from the hypodermis although normally they are of mesodermal origin. Miss Reed ('04) finds this to be true in the regenerating leg of the crayfish. Ost ('07) notes the same phenomenon in the regenerating antennæ of *Oniscus*.

It is recognized, then, that certain tissues originating normally from different germ layers may arise in a regenerating organ from the same germ layer. It would be at least possible that, although the nerve connections between the optic ganglion and the retinulæ arise as processes from the ganglion in the development of the normal eye, they might arise as processes from the retinulæ in the regenerating eye. As even in this case they would develop from the same germ layer although from different parts of it.

The possibility that the nerve connections may have arisen differently in the embryonic eye and in the regenerating eye is conceivable. Yet it seems that the evidence obtained from a comparative study of the normal adult eye and the regenerating eye suggests that the nerve processes develop from the retinulæ in the normal eye just as in the regenerating eye.

The preceding pages show that the development of the regenerating compound eye corresponds in a general way with the embryonic development of the compound eye. They also show that the observations made upon the regenerating eye do not agree entirely with those of any one worker upon the embryonic development of the compound eye of Arthropods. In many respects, however, there is a close similarity between the development of the regenerating eyes of *Palæmonetes* and hermit crabs and the process of

differentiation in the embryonic eye of the lobster as described by Parker (*loc. cit.*) Further, these observations upon regenerating eyes agree with those of Phillips upon the developing compound eye of the honey bee in regard to the order of appearance of the retinulae and cones, in the method of innervation of the ommatidia and in regard to the relation of the cones and rhabdoms. In the developing eye of the honey bee, however, Phillips finds the rhabdoms partially differentiated before there is any indication of the cones. On the other hand in the regenerating eyes of hermit crabs and *Palæmonetes* the cones are definitely formed before any rhabdoms can be recognized. The variations, however, which have been noted between the developing compound eye of the honey bee and the regenerating eyes of *Palæmonetes* and hermit crabs, cannot be regarded as fundamental. Such differences are perhaps not more marked than those that would be noted if the embryonic development of the same eyes were compared.

It is scarcely necessary to add that these observations on regenerating eyes are in several respects quite at variance with the observations of Kingsley and Patten, who find the rhabdoms developed from an inward prolongation of the cone cells, the cones formed as extracellular secretions and the ommatidia innervated by nerve processes coming from the optic ganglion and penetrating the rhabdoms and cones.

4 *Differences in the Regeneration of the Eye Among Palæmonetes, Crangon and Hermit Crabs*

Reference has already been made to the fact that certain differences in the regenerating eye appear among *Palæmonetes*, *Crangon* and hermit crabs. The rate of regeneration and the ability to regenerate varies greatly in these different genera although in the most essential particulars the regeneration of the ommatidia is similar. It has been seen that hermit crabs may regenerate an eye after the removal of half the optic ganglion. But neither *Palæmonetes* nor *Crangon* regenerate a perfect eye if the injury includes any part of the optic ganglion. It has also been seen that the differentiation of the ommatidia takes place more rapidly in

the hermit crabs than in either of the other forms. The only structure in the eye of the hermit crab which apparently does not regenerate perfectly is the basement membrane. This membrane, however, is but slightly developed in the normal eye. It is not strange, therefore, that it appears imperfect in the regenerated eye.

The regenerated eyes of the hermit crabs in these experiments have developed from a level below the basement membrane. In every case they present a clearer and more normal appearance than the regenerated eye of either *Palæmonetes* or *Crangon*. This is largely due to the fact that in the eye of the hermit crab there are no shapeless masses of old pigment scattered among the regenerated tissues as is usually the case in *Crangon* and *Palæmonetes*. The absence of the yellow accessory pigment cells in the eye of the hermit crab also tends to give to the ommatidia a distinct and orderly arrangement. The absence of this accessory pigment is, however, not due to incomplete regeneration. The normal eye of the hermit crab, unlike that of many Decapods, contains no accessory pigment cells. These pigment cells are very abundant in the eyes of *Crangon* and *Palæmonetes* and tend to make the ommatidia less clear.

The most significant difference between *Palæmonetes* and *Crangon* seems to be in the rate of regeneration after similar injury. External appearances would indicate that *Crangon* regenerates more rapidly than *Palæmonetes*. But a comparative study of the section shows that the reverse is true.

In almost every individual in the series of twenty *Crangon* operated upon the injury was slight. The wound healed rapidly, the animals moulted frequently and externally there was every indication that regeneration was rapidly taking place. An examination of the sections, however, shows that in none of them has there been any considerable regeneration. On the other hand, in each case much of the old injured tissue remained in a semi-broken down condition.

Sections of the eye represented in Fig. 8 show that one part of the eye had not been injured below the level of the outer reticular cells. The proximal ends of the retinulæ still remain intact although thirty-one days have elapsed since the operation. A

continuous hypodermis has not yet been formed. A considerable area between the cuticle and the outer retinulæ is occupied by a granular structureless mass. Just to one side of this area is a region in which none of the old ommatidia appear but in which there are new ommatidia almost completely formed. No more than five of these appear in any one section. These lie near the basement membrane toward the inner edge of the eye. Consequently these new ommatidia lie next the growing zone, always present in the eyes of young individuals as described in Section III. It is apparent then in this particular case that it is impossible to determine conclusively whether the new ommatidia are regenerated or normally developed ommatidia. In other cases, however, new ommatidia are found developing in positions where it is evident they are regenerating ones.

From the evidence obtained by an examination of a number of regenerating eyes of *Crangon* it seems that the rate of regeneration depends largely upon the rate of removal of the injured tissue. The failure of the old tissue to degenerate prevents the regeneration of new structures. Since the injured ommatidia, although they fail to break down for a considerable time at least, are incapable of regeneration in themselves. We should perhaps expect the cones to be incapable of any sort of regeneration for the cone nuclei have been destroyed and the constructive metabolic activity of a cell apparently depends largely upon the nucleus. The nuclei of the retinulæ, however, have not been injured and still retain much of their normal appearance. But there is no evidence that the retinulæ ever take part in normal regeneration.

A comparative study of the regenerating eyes of *Palaemonetes* and *Crangon* shows that the difference in the rate of regeneration is in reality largely a difference in the rate of degeneration of the injured tissues. In *Palaemonetes* the injured tissues usually break down rapidly and are quickly removed. In *Crangon* they persist indefinitely. Hence regeneration in *Palaemonetes* begins soon after the injury, and new ommatidia may be almost fully developed in shorter time than is required by *Crangon* for the removal of the injured ommatidia. The specific case of *Crangon* perhaps suggests that if all of the ommatidia had been completely

removed by the operation, that regeneration would have followed more rapidly. An inference which is supported by the observations of Zeleny ('05), who finds that in regenerating appendages of crayfish, an increase of the injury, increases the rate of regeneration. On the other hand, the same inference cannot be applied to the regenerating eyes of *Palæmonetes*; for as has already been pointed out for this form new ommatidia differentiate more rapidly when a part of the old ommatidia remain uninjured.

5 *Comparison of Normal and Regenerated Eyes*

It seldom happens that a regenerated eye appears altogether normal either from external or internal examination. Externally they frequently appear abnormal in shape and are always smaller than the opposite eye. These external abnormalities are, however, of no especial importance except in so far as they may indicate internal conditions. A common external feature which is suggestive of internal conditions is the irregular arrangement of the pigment masses. Internal examination shows that these masses are frequently remains of broken down retinulæ.

Besides the pigment remains of the old retinulæ, a number of other abnormalities may appear which make it difficult to interpret sections correctly. The arrangement of the retinulæ makes it difficult to group them into distal and proximal rows of nuclei as can be done readily in the normal eye. It is quite possible in cross sections to select ommatidia in which eight retinulæ, the typical number, can be counted but in other cases this number cannot be recognized owing possibly to the suppression of the eighth retinular cell, which is rudimentary in the normal eye. The difference in the length of normal and regenerated ommatidia is quite noticeable in many cases. The regenerated ommatidia are often much shorter. The new ommatidia might have grown to normal size, however, had the experiment covered a longer period of time.

The remaining significant difference between the normal and regenerated eyes is that in some regenerated eyes the optic ganglion is not complete. This difference appears only in the hermit crabs as these forms may develop an eye after half the optic ganglion has

been removed. In no case is there any evidence that the optic ganglion regenerates. Consequently this difference would remain unchanged.

B CASES OF ABERRANT REGENERATION OF OMMATIDIA

In study of the regenerating eye several cases of aberrant regeneration of ommatidia have come under observation. One case deserves especial mention. Fig. 36, *a* and *b* represent ventral surface views of an injured right eye and the normal left eye of a *Palæmonetes*. Judging both from the surface indications and an examination of the sections the right eye must have been cut off at a level corresponding approximately with the line *a-b* shown in the figure of the normal eye. A cut at this level would remove the upper two sections of the optic ganglion and injure the third. It would also cut across the heavy muscle band lying in the posterior part of the eye stalk.

The experiment covered thirty days, one moult taking place ten days after the operation. Casual surface examination was sufficient to show that a rather large amount of new tissue had formed and that a spot of pigment had developed on the ventral side of the stump. The growth of such a large amount of new tissue is quite unusual when so much of the ganglion has been removed.

A study of the sections gives additional information regarding this new tissue. In Fig. 56 the tissue lying between the periphery and the broken line extending from *x* to *y* represents approximately the amount of new tissue. Careful examination shows a difference in the character of the differentiation of the regenerated tissues in the different regions. This figure is from a section so near the dorsal surface that but little of the nerve tissue appears. Near the right side of the figure a conspicuous section of the old muscle band is seen. Just distal to the muscle band the new tissue is more dense and compact than in the remaining part of the regenerating tissue. This band of new tissue (*nt*) is composed of fibers extending inward from the periphery and joining end to end with the fibers of the old muscle band, thereby forming a contin-

uous band and reestablishing the broken connection between the muscle and the chitinous covering of the stalk.

Fig. 57 of the same series represents a section deeper in from the dorsal surface so that parts of the optic ganglion are apparent. A few scattered spots of pigment are also present. Here again the regenerated tissue shows differentiation into strands of fibers in the part lying beyond the remains of the old muscle band. In other regions there is a loose network of fibers with scattered nuclei. A difference, however, in the appearance of the nuclei in different regions of the regenerated tissue can be observed. From *a* to *b* the nuclei are small and inconspicuous, constituting uniformly granular masses and staining with but little more intensity than the fibers which extend inward from them. The nuclei lying between the points *b* and *c*, on the contrary, are conspicuous, stain deeply and are more than twice the size of those lying between *a* and *b*. Here also a number of nuclei are seen lying below the periphery which show a tendency to extend straight inward from the periphery. Fig. 58 shows the upper part of a section that lies so near the ventral surface that it is entirely outside most of the optic ganglion. In this figure no part of the muscle band appears. Conspicuous masses of pigment are present in this section as well as a great number of relatively large deeply staining nuclei. Since the eye was cut longitudinally from the dorsal to the ventral side the sections near the ventral side are approximately tangential, so that many of the nuclei that appear to be deep in from the periphery are in reality near to the surface. This needs to be kept in mind in interpreting the figures.

It is a conspicuous fact that many of the nuclei shown in Fig. 58 resemble in shape and appearance the reticular nuclei found in sections of regenerating eyes. Further resemblances between these and reticular nuclei are their tendency to stain deeply and the general direction of their long axes which is at right angles to the surface. These facts considered in relation to each other leave but little doubt that these elongated nuclei represent the reticular elements in a regenerating eye. At one point in Fig. 37 (*c.c.*) the rudiments of two crystalline cones have appeared. This is additional evidence that an eye is regenerating, imperfect and

abnormal as it may be. The pigment shown in this figure represents the maximum amount seen in any section. For the most part it presents no definite arrangement but lies in irregular masses clustered within a fairly well defined area. In a few sections, however, a part of the pigment shows a tendency toward a normal arrangement as if the pigment granules were contained within the processes of the reticular cells, and rudimentary ommatidia can be recognized (Fig. 59).

Any attempt to explain the phenomena presented by the eye under discussion may appear somewhat premature, since it presents a practically unique case so that but little data for comparison is available. In the first place this is the only well established case of any attempt of *Palaemonetes* to regenerate ommatidial structures after the removal of a large part of the optic ganglion. It seldom happens that any regeneration takes place from the eye stump of *Palaemonetes* when no more than half of the optic ganglion remains. Before attempting to explain the phenomena, therefore, it is well perhaps to examine the results of other observations that may suggest an explanation.

From the evidence obtained from the study of normally regenerating eyes the indications are that the first new regenerated tissue is largely of an indifferent character, *i. e.*, capable of giving rise to different structures, as determined by conditions more or less external to itself. It has been seen that in the regenerating eye the primary hypodermis gives rise to the cells which develop into the different structures of the ommatidia. In the earliest beginning of differentiation, if a cell divides so that the plane of division is at right angles to the surface the two resulting cells are hypodermal cells. If on the other hand the plane of division is parallel to the surface the inner one of the pair thus formed becomes a reticular nucleus and the outer one remains hypodermal in character. At this stage the only apparent difference between the two nuclei is in their respective positions. In later cell generations when the division plane is parallel to the surface the inner nuclei of the pairs formed become crystalline cone nuclei. Thus we have cone nuclei, reticular nuclei and hypodermal nuclei indistinguishable except for their relative positions. Apparently the subsequent

differentiation of each is conditioned by their relative positions in respect to each other, to the surface and to the old parts present.

That the relation of the old tissues to the regenerating tissues is a determining factor in the regenerating of the new structures has been maintained by several workers. Child ('04) who especially emphasizes the idea, says: "The fate of the new material must be regarded as depending essentially upon its relation to the old parts." That the regeneration of one structure may be dependent upon the presence of another has been shown by Lewis ('04). He found that a lens could be developed from any part of the ectoderm of a frog embryo by transplanting the optic vesicle and allowing it to come in contact with the ectoderm. In this case it appears that the actual contact of the two tissues constituted a determining factor and that the new conditions have arisen on account of the new relations of the two tissues.

From the instances referred to the following inferences may be drawn—first, newly regenerated tissue is largely indifferent in character; second, the differentiation of the new tissue is largely conditioned by its relation to the old tissue. The above inferences may be used in suggesting an interpretation of the special phenomena under consideration.

To begin, it is evident that the particular individual now being considered exhibited a more than usual degree of physiological and regenerative activity. In no other way could we account for so much more new tissue than is ordinarily regenerated by a stump of this length. The sections show that a large part of the new tissue still presents an undifferentiated appearance, although in certain regions differentiation has begun. Just above the cut end of the old muscle band (Fig. 35) the new tissue appears thickened, arranged in definite fibers and is apparently continuous with the muscle band suggesting that connections between the muscle and the chitinous covering of the eye had been reëstablished.

Sections passing through the stump near its ventral side show rudimentary ommatidia in process of development. Just why ommatidia should appear on the side of the old stump is not at first sight apparent. One possible explanation of this phenom-

enon has, however, suggested itself. The differentiation of new ommatidia appears to depend largely upon the reestablishment of connections between the optic ganglion and the new tissue. That such is the case is suggested by the fact that in regenerating eyes the reticular processes reach the optic ganglion before the cones begin to differentiate and before any pigment is deposited in the retinulæ. Further, according to Parker (*loc. cit.*) in the embryonic development of the lobster's eye from the earliest beginning of differentiation there is a connection between the ommatidial region and the optic ganglion and this connection is never lost at any stage in the development of the eye. Since the pigment appears in the retinulæ after they have formed connection with the ganglion its presence in this stump (Fig. 36a) may be an indication that the connections between the optic ganglion and the new tissues have been formed and consequently that further development of ommatidial structures has been initiated.

This suggested explanation does not of course give any reason why ommatidial development should begin on the ventral side rather than elsewhere. The following explanation is suggested. Cross and longitudinal sections of the normal eye stump show that the optic ganglion extends somewhat nearer the surface toward the anterior ventral side. On this account perhaps rudimentary ommatidia have developed on the ventral side first because the distance between the new tissue and the optic ganglion was shorter so that nerve connections were more quickly established in that region than elsewhere.

Other cases of aberrant regeneration have also come under observation. These, however, have been produced apparently by pathological conditions. One or two of the more interesting cases will be described. Fig. 19 represents dorsal surface views of a *Palæmonetes* eye thirty-one days after the removal of the upper part of the ommatidial portion. Eight days after the operation a moult occurred. A second moult occurred fifteen days later and it was seen that the greater part of the ommatidial structures had disappeared. Fig. 19 shows a mass of pigment just distal to the optic ganglion. Above this pigment there is a considerable area of transparent tissue which shows no external evidence, however, of

being differentiated into ommatidia. Sections of this eye show a considerable development of abnormal pigment. At one side are seen a limited number of regenerating ommatidia that have failed to differentiate normally. The arrangement of the abnormal pigment is of a character frequently seen in short stumps but not generally found where the injury, as here, has not involved the optic ganglion. The probable cause of this abnormal pigment deposition will be considered in another section. The point of chief interest here is that we find two sorts of development going on side by side, one region developing normal structures and a contiguous region developing abnormal structures.

Another similar, though rather more exaggerated, case is furnished by a small hermit crab (Fig. 25). The eye had been injured before the animal was brought into the laboratory. No moult, however, had taken place since the injury. About two weeks after being brought into the laboratory the crab moulted and three days later was killed. Fig. 25 represents the ventral view both of the injured and uninjured eye three days after the moult. It is seen that the injury involved the whole ommatidial portion and apparently a part of the optic ganglion. The distal surface of the regenerating eye presents a very irregular outline. On the inner border a peculiar protuberance has developed and two separate pigment areas are apparent. The upper of these two areas suggests that small ommatidia have been regenerated. Externally the lower pigment mass suggests no probable explanation of its character.

Sections of this eye show a number of interesting points. In the first place it is demonstrated that the upper and smaller pigment area belongs to the retinulæ of small but almost completely developed ommatidia. All the structures of a typical ommatidium have been differentiated with the exception of the corneal facets and the spindle shaped enlargement at the base of the rhabdom. The lower pigment area is an abnormal pigment deposition similar to the preceding case.

The protuberance developed on the inner border of the eye seems also to be formed of abnormal tissue. Its interior is entirely made up of a loose irregular network of tissue containing a number

of faintly staining nuclei. These tissues resemble very closely the depigmented tissues of the abnormal pigment masses. This resemblance suggests that possibly the two abnormal appearances have had a common origin.

A case partially resembling the one just described was observed in a green shrimp, *Palæmonetes viridis*. The original injury consisted in the removal of a small part of the top of the eye. The eye was operated upon August 1. On the ninth of the same month the animal moulted and was preserved. Figs. 17 and 18 represent dorsal and ventral views of the injured eye. The dorsal surface of the whole eye is shown in Fig. 17. Fig. 18 represents the distal end of the eye from the ventral side and under greater magnification. A pigmented mass similar in general outline to the reticular area in the normal eye is visible through the transparent outer tissues. Distal to the pigmented portion is a considerable area of transparent tissue with flecks of pigment scattered through it.

The distal contour of the eye is irregular because of a swelling or protuberance similar to the one on the hermit crab's eye previously described. This eye also shows an unusual development of new tissue considering the time in which it was produced.

Sections of this eye show that the optic ganglion had not been injured, that not all of the ommatidia had been removed and that a considerable part of the old pigment remained. The ommatidia that were left have almost completely degenerated, however, and the whole distal portion of the stump is filled with a complicated network of faintly staining cells. There is also absolutely no regularity in cellular arrangement, as is seen in normally regenerating eyes. For the most part the nuclei are scarcely distinguishable from the cell-body. Although here and there are scattered nuclei which stain more deeply. There are evidences in some cases that these are nuclei of disintegrating ommatidial structures. Some sections show remains of old cones associated with the darkly staining nuclei. Comparison of others of these deeply staining nuclei with the nuclei of partially depigmented cells shows a similarity between the two which suggests that the former belong to cells in which pigment secretion has lately begun.

It is evident that most of the pigment masses present are the

remains of the old ommatidia although they are greatly scattered through the new tissue. A few dense cysts of new pigment, however, have been formed and other pigment secreting centers have begun to appear. From these observations it seems apparent that had the animal lived the entire mass of tissue sooner or later would have been densely packed with pigment cysts and that very probably new eye structures would not have regenerated. For we have seen in the preceding two cases that an abnormal secretion of pigment stopped, apparently, ommatidial regeneration after it had begun. It does not seem too much to assume then that in this case normal regeneration of tissues would have been precluded by such an abundant development of abnormal tissue.

C EYE STUMPS THAT SHOW AN ABNORMAL DEVELOPMENT OR NO REGENERATION

The instances described in the preceding section apply particularly to those unusual types in which the ommatidia have begun to regenerate and this process has been more than balanced by opposing factors. This leads naturally to a consideration of cases in which there is either no regeneration or only an abnormal development of pigment.

1 *Abnormal Development of Pigment*

Most of the examples of abnormal pigment secretion were afforded by *Palæmonetes* in which the optic ganglion was more or less injured. Usually in any of the forms studied eye stumps that contain no more than half of the optic ganglion show no normal regeneration aside from the cuticle and hypodermis. Any attempt to regenerate other tissues produces either scattered strands of connective tissue or abnormal masses of pigment. These pigment masses most frequently appear collected in nodules or cysts and are usually enclosed in a sort of connective tissue sheaths. Fig. 43 represents an eye stump of *Palæmonetes*, showing one of these pigment depositions. Fig. 72 shows an outline section through this stump from which the relation of the pigment to the normal tissues can be readily made out. Fig. 43 shows in detail the

appearance of the pigment outlined in Fig. 72. An examination of Fig. 73 shows that the deposition of pigment appears to begin at several centers. These centers gradually increase in size. There also seems to be a tendency for the several centers to fuse with each other. It is further seen that the pigment cells or masses vary from very large to very small areas.

A study of depigmented sections suggests that these smaller pigment bodies arise in one of two ways: first, by an out-pocketing of the cytoplasm, which after becoming distended with pigment separates from the parent mass and second, by an unequal division of the cell. It is possible that the latter is the true method for all cases. But it was not possible to determine this point with certainty. When a pigment cell has become gorged with pigment the nucleus is much changed and distorted. And even after the most thorough depigmentation it cannot always be identified. Consequently it may be that the smaller masses, in which no nuclei are visible, are not mere masses of cytoplasm that have been constricted off but are the result of unequal cell division. Fig. 75f shows a small group of depigmented pigment bodies. In the larger masses nuclei are visible. In the smaller bodies nuclei cannot be determined with certainty.

The amount of pigment within a cell varies. Some cells contain only a few scattered granules while others are so completely filled that they appear to be black homogeneous masses. In these more densely filled cells the pigment appears to have fused into solid brittle masses that can be crushed like starch grains. *g* in Fig. 75 represents one of these masses after it has been crushed.

The pigment is dissolved from the sections with the greatest difficulty. Mayer's chlorine method was generally used for this purpose. But in removing the cyst-like depositions of pigment it was found that alternate treatment with the chlorine method and with one-twentieth per cent KOH in 70 per cent alcohol gave equally as good and more rapid results. Even with this treatment twelve to twenty-four hours were required to remove the pigment from sections 6μ thick. Frequently this failed to dissolve the dense pigment masses. In Fig. 75 the dense, crushed pigment mass *g* lies in the same section with the group of depigmented

cells shown in *f*. It frequently happens that not all of the tissues included in what may be regarded as a single pigment region are pigmented (Fig. 73). The unpigmented tissues, shown in the area represented in this figure, contain but few recognizable nuclei. Here and there are cells that show a few pigment granules and occasionally small groups of such cells. These facts together with the general appearance of the tissue suggest that eventually the entire area might have become packed with pigment.

Figs. 29, 32, 44 represent eye stumps of *Palæmonetes* that show somewhat different types of these abnormal pigment formations. Fig. 32 presents a rather unusual type. Externally the pigment appears as thickly scattered granules instead of a dense black mass as in most cases. Sections of this stump show a small quantity of new tissue lying at the distal end and alongside the nerve stump. The cells composing the new tissue are closely packed, large, granular, and their nuclei do not take up iron hæmatoxylin at all. Along the side of the eye stump a number of small pigment cysts appear but for the most part the cells of the new tissue are not yet densely pigmented. Many of them, however, show numerous pigment granules. This particular specimen shows less of the connective tissue-like, fibrous network than is usually found in the pigment areas. Apparently this stump shows an early stage in abnormal pigment secretion. The other two cases figured show dense masses of pigment. Fig. 44 presents a single compact mass. In each case sections show the pigment arranged in the characteristic cysts, such as are seen in Fig. 73.

One additional fact of interest is shown in Fig. 44. The pigment cysts in this case do not lie wholly above and distal to the remains of the optic ganglion but are embedded in the end of the optic stump. Apparently the upper part of the ganglion stump has degenerated and given place to the pigment. This is not an unique instance as several other stumps have presented a similar phenomenon. There was one case in particular in which there were several small pigment cysts embedded in different portions of the remains of the optic ganglion. The ganglion, in this case had almost entirely degenerated, apparently. This animal had been preserved in alcohol, however, and it was consequently impossible

to determine just how much of the abnormal appearance of the tissue was due to degeneration before the death of the animal and how much was due to disintegration after its death.

It is evident that this sort of pigment development, whatever may be its cause, does not belong to the normal regeneration of an eye. Further, it appears probable that the causes leading to its formation are of such a nature that they inhibit the true regenerative process. The last two cases described in the preceding section furnish evidence of this. In the eyes shown in Figs. 19 and 20 regeneration of normal ommatidia had begun but was limited by some opposing factor. These causes not only inhibit the true regenerative processes after they have begun but it is also probably true that they even prevent true regeneration from beginning. All the comparative evidence that we have indicates that in the case of the *Palæmonetes viridis* previously described (Fig. 17) a new eye would never have developed. The whole distal end of the stump was filled with a mass of abnormal pigment depositing cells. Although this case is striking it is not exceptional. Similar conditions have been found in varying degrees in other eye stumps. There is sufficient similarity in all the cases of abnormal pigment deposition to indicate that they have in certain respects a common cause.

It is important to point out some of these similarities in greater detail. A striking resemblance exists between the broken down retinulæ of an injured eye and the pigment secreting cells. In the early stages of the disintegration of the ommatidial structures the nuclei of the retinulæ frequently become separated from the reticular processes. Each nucleus becomes surrounded by a rounded mass of cytoplasm which apparently has no connection with other structures. The nuclei become polymorphic and not infrequently appear divided. As the disintegration proceeds these rounded nuclear cells usually disappear, but, as mentioned in a preceding section, the broken down masses of pigment remain. These rounded remains of the retinulæ can be identified from a few hours up to sixteen days after the injury. They are always seen a few hours after the injury although they may not always be present in eyes examined in a week to two weeks after the opera-

tion. This shows that in some cases they disintegrate much more rapidly than in others. In some eyes examined twenty-five to thirty-five days after the operation similar rounded cells with polymorphic nuclei are found in numbers, increasing by amitotic division. In still other cases, cells of this character containing pigment granules are found.

Fig. 75*a, b, c, d, e, f, g* represents a series of groups of these rounded cells with polymorphic nuclei. These groups were taken from crayfish, hermit crabs, Crangon and Palæmonetes, representing in all seven species. The first three groups, *a, b* and *c*, show the appearance of breaking down retinulæ, seventeen hours, thirty-nine hours and sixteen days, respectively, after the injury; *d, e* and *f* show the secretion of abnormal pigment as found in eyes ten, twenty-three and sixty-seven days respectively after the injury; *f* represents a group of depigmented cells that were so filled with pigment that without depigmentation no structures were visible. An examination of this series cannot fail to show the similarity between the breaking down retinulæ and the pigment secreting cells. Particularly is this so if it is remembered that, except *a* and *b*, no two groups are taken from the same species.

These facts taken together have suggested that the immediate cause of the pathological pigment secretion is the abnormal activity of old retinulæ which have not completely broken down. It has already been mentioned that after an eye has been operated upon the pigment from the injured retinulæ frequently becomes greatly scattered among the other tissues. Not only does the reticular pigment become scattered but in some cases the rounded reticular cells, also, are found considerable distances down the stalk and on the side opposite the injury. These instances were observed in eyes examined from fifteen to twenty days after the injury. It seems probable that some of these metamorphosed reticular cells become embedded in other tissues, then later divide amitotically and begin to secrete pigment. The nodules of pigment previously described are the result. In some cases the multiplication of these pathological cells takes place rapidly so that large areas are occupied by them. Fig. 70, which is from a section of the eye shown in Fig. 20, represents such a case. A

relatively large amount of new tissue was regenerated by this eye, and sections show that normal regeneration had begun (Figs. 68, 69). A new hypodermis was completely differentiated and on one side the differentiation of ommatidia was taking place (Fig. 69). The greater part of the new tissue was made up, however, of cells of a character known to be abnormal. The hypodermal cells are practically the only cells that appear normal. With the exception of a small area on one side almost the whole of the interior is filled with rounded cells containing polymorphic nuclei. Fig. 71 represents a part of Fig. 70 more highly magnified and showing the structure in greater detail. A comparison of these two figures with the series shown in Fig. 75 cannot fail to show a striking similarity.

Altogether there is strong evidence that the failure of the old retinulæ to disintegrate completely is the immediate cause of abnormal pigment deposition, in many cases at least. Further, there is some evidence that regenerating retinulæ may sometimes become involved in the abnormal secretion of pigment. Group *d*, Fig. 75, represents a probable case of this sort. The group was taken from the regenerating part of the eye in a region where there is positive evidence that some normal regeneration is taking place. A few of the cells in the group still show but few pigment granules and show elongated nuclei, characteristic of regenerating retinulæ (Fig. 75*d*, *ret.n.*)

The appearance of the network of tissue with which many of these pigment nodules are associated still remains to be accounted for. Evidences which point to the origin of this are not so numerous as are the evidences that the old retinulæ form the centers for the pigment secretion. In some cases these pigment nodules are found embedded in the hypodermis of the eye stalk, in other cases in the membrane surrounding the optic ganglion. In such instances it seems probable that the fibrous network supporting these nodules is due to the hypertrophy of the normal tissue immediately surrounding the pigment deposits. In those cases, however, where a great mass of this fibrous network developed it seems to have had a different origin. There are three particularly striking instances of the unusual development of this abnormal

tissue, each furnished by a different form. Sections taken from a crayfish eye fixed sixty-two and a half hours after the operation show a condition that is apparently an early stage in such development. At this stage the network is not yet compact and is found in chains of elongated cells, showing nuclei dividing amitotically. These chains of cells run in all directions but do not appear to develop from the hypodermis. Some sections show these chains extending from the injured retinulae which still surround the remains of the old cones (Fig. 74). This suggests that the old reticular cells are undergoing rapid multiplication.

The chains of cells found in the crayfish eye differ in the following respects from the network of abnormal tissue found in *Palæmonetes viridis* and hermit crab, shown in Figs. 17 and 25. In the cases of the hermit crab, Fig. 25, and of *Palæmonetes viridis*, Fig. 17, the cells constituting the network are no longer recognizable as chains and the nuclei no longer stain deeply nor appear to be dividing. These differences may be accounted for by the following facts. First, the whole available space in the stump of the eye was completely filled with the network in the eyes of hermit crab and *Palæmonetes viridis* and the chains of cells had become so completely interwoven that their original character could no longer be recognized. Second, it is probable that the nuclei no longer stain deeply because the cells have ceased active division. It has been shown that the cells cease to divide actively soon after the secretion of pigment begins. In these cells the secretion of pigment had begun.

Assuming that these apparent differences have been accounted for we may now turn to their likenesses which suggest a similarity of origin. The most suggestive likeness is that in each of the three forms the abnormal tissue appears to have developed outward from the base of the wounded area rather than inward from the periphery. The most striking evidence of this is the fact that masses of old pigment appear near the periphery as if they had been carried outward by the growth of the new tissue. These abnormal tissues, in the case of hermit crabs and *Palæmonetes*, lie close against the cuticle and several layers of the cells are flattened as if they were the oldest cells and had been pressed against the

cuticle by the multiplication of the cells beneath. In these cases a true hypodermis is not distinguishable. These facts suggest that the migration and pathological development of the old retinulæ are responsible for most if not all of the cases of abnormal pigment deposition. This of course does not explain what induces this pathological development.

The initial cause of this development, in the cases where an abundant network of tissue has developed, was perhaps due to some infection at the time of the operation. This is suggested by the fact that, in the crayfish eye described above, a great deal more tissue had developed abnormally in sixty-two hours than is usually developed normally in ten days or two weeks, and by the fact, also, that in the eye of *Palæmonetes viridis* a very unusual amount of new tissue had developed during the first nine days after the injury. The more frequent cases in which the pigment secreting cells appear as rounded cells, containing polymorphic nuclei similar to the disintegrating retinular cells, seem to be produced by causes somewhat different. In some specimens examined some time after the injury these cells show no signs of rapid multiplication. It seems probable that these cells are old retinulæ that have retained one of their characteristic functions, the secretion of pigment. Since it is an observed fact that the old retinulæ become metamorphosed and wander to different parts of the stump where they have been found dividing amitotically.

While the above facts are strongly in favor of the conclusion that the abnormal pigment-secreting tissue is due to the development of old retinular cells yet the proof is not absolute. A series of stages of this development, not more than two days apart, would have to be examined in order to be certain of the absolute truth of this tentative conclusion.

2 *Eye Stumps that Show No Regeneration*

It now remains to consider the other phase of the subject outlined in this section; namely, those cases in which there is no regeneration further than the healing of the stump. A number of these cases present anomalies in that there is no apparent reason

for their failure to regenerate. There were among the hermit crabs several parallels between those that regenerated an eye and those that did not, so far as conditions were concerned. In a series of fourteen hermit crabs that had the ommatidial portion of the eye removed five regenerated an eye and nine did not. All were kept as nearly as possible under the same conditions. The part of the eye removed in the original operation was about the same for each individual. All were operated upon at the same time in the same way. Some of those that regenerated an eye and some that did not moulted upon the same day after the operation. Consequently the physical condition of these specimens were apparently similar.

Compare Fig. 13 and Fig. 21. Each of the hermit crabs from which these figures were taken moulted twelve days after the operation. The hermit crab from which Fig. 13 was taken was killed at the end of thirty-eight days and the other at the end of sixty-seven days. The latter lived nearly twice as long yet it shows no signs of regeneration. More of the optic ganglion remains in the stump shown in Fig. 21 than in Fig. 13. Again, compare Fig. 15 with Fig. 21. The two crabs from which these figures were taken were operated upon at the same time, moulted approximately upon the same dates and were killed sixty-seven days after the operation. The stump shown in Fig. 21 shows no regeneration while the one shown in Fig. 15 has regenerated an eye perfect in all of its details.

The number of cases might be multiplied but these given are sufficient to show the parallels presented by individual cases. Instances of this sort are confined in great part to hermit crabs. A number of shrimp, however, failed to regenerate even when the optic ganglion was not injured. The same is true for Crangon which in several instances failed to regenerate normally even after the removal of only a small part of the eye.

In most cases sections of such eyes that did not regenerate show no recognizable pathological conditions. In the case shown in Fig. 21, however, there was found what seemed to be the beginning of pathological pigment development. Externally there were no signs of pigment formation. The regenerated tissue consisted of a

heavy cuticle, a hypodermis and some loose strands of tissue extending from the hypodermis to the distal end of the stump of the optic ganglion. Grouped at the end of optic ganglion stump and scattered in the loose tissue above it were a few cells of the characteristic pigment-secreting type. But none of these cells had yet become densely filled with pigment (Fig. 75*e*). It seems rather improbable that so little abnormal tissue in which scarcely any secretion of pigment had taken place could have been the sole cause in the prevention of normal regeneration. Particularly is this true when it is remembered that instances have been observed in which practically complete ommatidia were regenerated in eyes containing great masses of abnormal pigment (Figs. 19, 25).

VI REGENERATION AFTER REMOVAL OF THE GREATER PART OR ALL OF THE OPTIC GANGLION

There now remains for discussion those cases in which the whole or most of the eye stalk was removed and consequently either all or the greater part of the optic ganglion. *Palæmonetes*, *Crangon* and hermit crabs will each be considered independently since the differences presented by them are such as to require separate treatment.

A HERMIT CRABS

Of a total of sixty hermit crabs operated upon twelve died as a result of the operation, a loss of 20 per cent as against 55 per cent of the *Palæmonetes* after a similar operation. Thirty-six of these remaining crabs moulted from one to three times and lived from twenty-three to one hundred and ninety-four days. These thirty-six crabs fall into two groups: those that regenerated an antenna-like appendage in place of an eye and those that showed no particular regeneration.

1 *Regeneration of Heteromorphic Appendages*

Ten crabs in all regenerated an appendage from the old eye stump. In but one case was more than thirty-two days required for the appendage to become apparent. All of these appendages are very small none exceeding in length the normal eye stalk. It

is probable, however, that they would have increased both in diameter and length had the experiment covered a longer period of time. None were distinguishable before the occurrence of a moult. In each case recorded the appendage appeared after the first moult. Most of these appendages were definitely segmented after the first moult, in some instances several segments being developed within twenty-one or two days. But none show any indication of being divided into parts corresponding to the exo- and endopodites.

Figs. 23 and 30 show two appendages that were present twenty-one and twenty-two days respectively after the injury. Neither appendage exceeds in length the squame at the base of the normal eye stalk which measures but little more than one-fourth of the whole length of the normal stalk. One appendage bears a considerable number of large tubular hairs. The other shows none whatever. Each is seen to consist of several segments. Five segments are distinctly visible in Fig. 23 while in Fig. 30 there are six or seven though they are not distinctly differentiated. In Fig. 30 the appendage projects outward at a broad angle. Fig. 23 is unique in that it curves in toward the median line and suggests in its general shape and position the squame at the base of the opposite eye. The bifid tip of this appendage is probably due to some injury that occurred at the time of the moult. This explanation is suggested by an examination of the specimen.

Figs. 34 and 45 represent two other appendages that appeared twenty-nine and thirty-two days, respectively, after the injury. These types differ somewhat from the preceding two. It is to be noted in both cases that the original operation did not include the squame at the base of the eye. This is a good indication that at least a part of the proximal segment of the optic ganglion was left. The specimen shown in Fig. 34 was sufficiently transparent so that the optic nerve could be observed extending into the base of the segmented appendage. The specimen from which Fig. 45 was taken was fixed in Flemming and the consequent darkening of the tissues prevented an accurate determination of the length of the stump of the optic nerve. But the nerve stump could be seen extending well into the base of the new appendage.

The appendage shown in Fig. 28 developed in twenty-four days with the intervention of one moult. It is of interest because of the indications that the optic nerve has extended through almost the entire length of the regenerated appendage. It is also of interest because of the ganglionic swelling that appears to be associated with the nerve in its distal half.

Fig. 41 shows an unique type in that the appendage is curved closely back until the free end almost touches the head. Although this appendage is made up of several segments it was rigid from its first appearance.

The remaining examples of these appendages are of approximately the same character as those figured. They belong chiefly to the type shown in Fig. 34, except that two of them show a larger number of tubular hairs. One of these belongs to a specimen that moulted twice and was not killed for sixty-seven days after the operation. The regenerated appendage shows but little advance over those that were fixed at the end of half that time. It is still no longer than the normal eye stalk and shows no greater number of sensory hairs than are seen in Fig. 30. The additional facts obtained from an examination of the sections will be referred to at the close of this section in the general discussion of their significance.

2 Cases that Show No Especial Regeneration

As was stated above out of the thirty-six crabs that moulted one or more times only ten developed heteromorphic appendages while twenty-six showed no particular regeneration. The proportion is a little more than 30 per cent to a little less than 70 per cent in favor of those that showed merely a healed over stump.

The stumps that show no actual regeneration present a variety of shapes and characters. None of them, however, show any signs of pathological pigment development. From all appearances the failure to regenerate in most instances was due to a lack of sufficient regenerative activity to produce the new tissue necessary. In some cases where the eye was taken off even with the head the wound healed over leaving a smooth surface, not so much as a slight elevation marking the former position of the eye. In

most instances, however, a longer or a shorter stump remained. It is impossible to determine by surface examinations how large the stump was originally for it decreases in size after the operation. Sometimes the stump of the optic nerve and ganglion shrinks to two-thirds of its original volume. Fig. 31 shows a short rounded stump which evidently contains a part of the proximal segment of the optic ganglion. The stump, originally as broad as the base of the opposite eye, has, after one moult twenty-three days after the removal of the eye, shrunk to one-half of the original mass. The remains of the optic nerve seem to come flush against the end of the stump, showing that no new tissue has been developed distal to it. Fig. 39, thirty-two days after the injury, shows a stump more than one-third the length of the normal eye. Yet sections show no indication that any definite structure is being regenerated. It is useless to multiply figures on this phase of the question. They only serve to show how completely is lacking any indication of regeneration.

The following table will serve to show that time cannot be considered the chief factor in regeneration.

No. of specimen	Experiment begun		Closed	Days		Moult	Regeneration
1	October	16	June	30	106	one	none
2	November	27	June	48	158	one	none
3	October	16	April	2	194	one	none
4	May	26	June	16	21	one	segmented appendage
5	May	26	July	4	39	one	none
6	July	9	September	3	56	one	none
7	July	9	August	3	25	one	segmented appendage
8	July	9	August	16	39	one	segmented appendage
9	July	9	September	14	67	one	none
10	July	9	August	6	28	one	segmented appendage

The last five examples given in the preceding table are taken from the same series. Evidently the conditions here were more favorable than usual. The original number of the series was twenty-five. Six of these died either from the effects of the operation or soon afterwards. Of those remaining five others were lost through an accident. Out of the fourteen for which there is a

complete record nine developed heteromorphic appendages and all of them within thirty-three days. Two of the remaining five, which moulted at the end of twenty-one days and then died, might perhaps have developed an appendage had they lived through a second moult. Each of them showed a very small bud where the eye had been removed.

B CRANGON

1 *Regeneration of Heteromorphic Appendages*

In some respects Crangon appears to be a favorable form for experimental work. They are less disastrously affected by the operation than the others worked upon. The entire eye was removed from twenty-two Crangon and not one of the number died from the effects of the operation.

This entire number was of the same series. The experiment covered a period of thirty-three days, August 3 to September 4, inclusive. During that time with one exception each individual moulted at least once and fourteen moulted a second time. Three of those that moulted but once were eaten by their comrades soon after the moult. The evident hardness of the Crangon and the frequency of the moults would seem to be favorable conditions for regeneration.

Results, however, show only one individual that regenerated a heteromorphic appendage, the others showing no regeneration. Fig. 38*a* and *b* shows surface views of this one regenerated appendage. The animal which developed it moulted on the fourth day after the operation. At that time there was no evidence of regeneration. Seventeen days later another moult occurred and an appendage of six segments, with sensory hairs near the tip, appeared. The appendage measures four-fifths of the length of the eye on the opposite side and projects forward at the same angle. The outline of the optic nerve can be seen extending through the proximal half of the appendage.

2 *Cases that Show No Especial Regeneration*

Twenty-one out of twenty-two Crangon showed no regeneration. Four of these died within nine days after the operation and so

perhaps should not be counted either way. There were then seventeen negative cases against a single positive case.

The eye stalks of Crangon are very short and the sections of the optic ganglion are crowded very close together, and extend well into the base of the stalk. Hence it not infrequently happened that a part of the ganglion remained in the stump. A number of these stumps have been sectioned and none of them show any regenerated tissue except the hypodermis and cuticle. Spots of pigment are often seen at the end of the stump but since the whole stalk of the normal eye is heavily pigmented this does not seem to be significant. Figs. 24, 37, 40 and 42 show a variety of appearances which the stumps presented. The accompanying table shows the number of moults which occurred.

No. of specimen	Experiment begun	First moult	Second moult	Third moult	Date of death	Regeneration
8	August 3	August 9	August 21	none	September 2	none
10	August 3	August 10	August 22	none	September 4	none
13	August 3	August 12	August 28	none	August 28	none
15	August 3	August 4	August 13	August 19	September 2	none
17	August 3	August 14	August 29	none	September 4	none

In some cases the eye stump is extremely short while in others it is longer so that a part of the ganglion remains. All of the specimens included in the table except No. 17 have been sectioned but none of them show any signs of regeneration. Sections of No. 10 show that nearly half of the optic ganglion was left but no regeneration is taking place. A very much folded and wrinkled cuticle with short hairs projecting from it covers the stump. Even No. 8 (Fig. 37), short as it appears, is found to contain the proximal end of the optic ganglion. In this case the stump has merely healed over but no new tissue has developed. In several other instances not shown in figures the eye had been totally removed so that not even a short stump is visible. In most such cases the cuticle is wrinkled over the spot where the eye had been. The wrinkles and folds on some of the stumps figured shows the common tendency. These folds are chiefly due probably to the shrinking of the inner tissues of the stump.

C PALAEMONETES

Out of nearly three hundred *Palaemonetes* not a single individual regenerated any sort of an appendage when all or nearly all of the optic stalk was removed. It is true that more than 50 per cent of them died from the operation or soon after. Often half or two-thirds of a series died within twenty-five or thirty minutes after the operation, and in some instances the proportion was still greater. (See Table I.) *Palaemonetes* were by far the least resistant of any of the forms operated upon. There were, however, over sixty individuals that lived from twenty to one hundred and twenty-four days and moulted from one to three times.

Considering the results of these experiments it may be said that *Palaemonetes vulgaris* does not regenerate an antenna-like appendage in place of an eye. Herbst would, perhaps, insist that these results were due to a lack of time or to a failure to remove all of the optic ganglion. This latter objection in many cases could not be urged. The eye stalk in *Palaemonetes* is long and the optic nerve extends well into its base. And in these experiments the eye was so completely removed that not even the vestige of a stump remained. Consequently there was no possibility of leaving any part of the optic ganglion. Part of the brain even was removed with the eye in two series. In regard to the other objection naturally there is no positive proof that results might not have been different in a longer period of time. There are strong reasons, however, for believing that time would have made no essential difference. Chief among these reasons is the fact that in the regeneration of any other organ *Palaemonetes* needs but little more time than the hermit crabs and less time than *Crangon*. In three parallel series of experiments upon the regeneration of the first antenna after its total extirpation it was found that *Palaemonetes* regenerates a first antenna as quickly and as perfectly as either *Crangon* or hermit crabs. In another parallel series of experiments upon the regeneration of the second antenna it was found that *Palaemonetes* regenerates this appendage rather more rapidly than either hermit crabs or *Crangon*. *Palaemonetes* may regenerate a first or second antenna in about thirty days. Neither

hermit crabs nor Crangon regenerate these appendages in less time. In the regeneration of a functional eye it was seen in a previous section that hermit crabs regenerate rather more rapidly than Palæmonetes but that Palæmonetes regenerate more rapidly than Crangon. Palæmonetes may regenerate a functional eye in thirty to thirty-five days. It is seen, therefore, that appendages are regenerated by Palæmonetes in approximately the same time as they are regenerated in hermit crabs and Crangon. Consequently it does not seem to be assuming too much to express the conviction that a greater amount of time would have made no essential difference in the results of these experiments in which the entire eye of Palæmonetes was removed.

Below is a brief table showing results obtained by removing the entire eye of Palæmonetes. This table does not include all the individuals of any one series but it is entirely representative.

No. of specimen	Experiment begun		First moult	Second moult	Experiment closed	Days	Regeneration
1	November	5	November 27	December 30	February 3	90	none
2	November	9	November	January	March 13	124	none
3	November	9	November	January	February 12	95	none
4	January	1	January 15	February 7	February 24	55	none
5	January	1	February 20	none	February 28	59	none
6	March	5	April 24	none	April 24	50	none
7	April	19	May 5	June 8 & 25	July 3	71	none
8	May	10	June	none	July 4	55	none
9	July	10	July 26	August 6	September 2	54	none
10	July	10	July 27	{ August 9 September 6 }	September 14	66	none
11	July	20	July 30	none	August 18	29	none
12	July	10	July 18	July 31	August 3	24	none
13	July	20	July 30	August 14	August 15	27	none
14	July	20	July 30	August 10	August 11	22	none
15	July	20	July 24	none	August 20	30	none
16	July	30	August 8	August 27	September 4	36	none

It will be seen that Palæmonetes have been under observation practically every month in the year. The results in each instance are negative. Fig. 35*a, b, c* represent some of the stumps that show new tissue distal to the nerve stump. Most of the cases, however,

regardless of the time of the experiment and the size of the stump, are similar to the one shown in Fig. 33, No. 13 in the table. Fig. 33 shows the nerve stump flush against the healed end. The indications from sections and surface views are not such as to lead one to expect that further regeneration would have ever taken place. Fig. 26 represents the only stump that even suggests the development of a heteromorphic structure. As the table shows this specimen lived only twenty-four days, during which time it moulted twice, and regenerated the tiny mass of new tissue represented by the darkly stippled portion of the figure. The eye was completely removed, the cut coming at the level of the attachment of the eye to the head, represented in Fig. 26 by the line *a-b*. Fig. 27 represents a more highly magnified view of the stump.

The remaining figures in the series, Fig. 35*a, b, c*, show the maximum regeneration, yet in none of these cases did the experiment cover more than thirty days. Apparently regeneration in most cases proceeds to the forming of the hypodermis and cuticle, which may be extended slightly beyond the nerve trunk by loose strands of connective tissue, and then stops. Fig. 35*c* shows more than the usual amount of new tissue. The line *a-b* represents the level of the union of the eye with the head. The unshaded central part of the eye stump shows the remains of the optic nerve; the shaded peripheral portion shows the new tissue. Neither sections nor surface examinations give the slightest evidence of the regeneration of nerve fibers, or of any special differentiation of the regenerated tissue.

D THE HISTOLOGY OF THE HETEROMORPHIC APPENDAGES

The microscopic structure of the antenna-like appendages has not been considered in great detail because suitable material has been wanting. In the whole series of experiments only ten hermit crabs and one Crangon ever regenerated a heteromorphic appendage in place of the excised eye. Several of these died and were preserved in alcohol. From such material no detailed results were obtainable. Again, the only sections of any particular interest and value are longitudinal ones. These heteromorphic append-

ages were so small and so curved that it was almost impossible to obtain satisfactory longitudinal sections.

A few points of interest, however, have been observed. These for the most part serve to corroborate the observations of Herbst rather than to add to them. An examination of the appendages in toto show that the old optic nerve either extended as a nerve trunk through the greater part of the length of the regenerated appendage or that other structures were developed in the new appendage which appeared to be continuous with the old optic stump (Figs. 30, 34, 38). Sections confirm the observations made from surface examinations. A large number of intermediate stages would be necessary, however, to determine whether the regeneration of the nerve trunk had been from the optic nerve stump outward or whether peripheral regeneration had developed nerve fibers inward which unite with the optic nerve stump. The fact, however, that the nerve trunk appears more distinctly differentiated in the proximal part of the appendage than in the distal may probably be regarded as an indication that the regeneration proceeds from the proximal end outward.

Sections of these appendages show that the interior is chiefly occupied by nerve cells and fibers. The nerve fibers appear to be continuous with the nerve fibers of the old optic nerve stump. The nerve cells are grouped into ganglion-like masses which are scattered pretty generally through the length of the appendage. The brain sheath is continuous with the loose fibrous sheath which envelops the mass of nerve cells and fibers.

Fig. 77 shows a somewhat diagrammatic section through the brain and the proximal end of a heteromorphic appendage that developed within sixty-seven days after the operation. It was necessary to combine two sections in order to show the continuity of the optic nerve with the nerve trunk of the appendage. There can be no doubt, however, that they form a continuous structure. One feature is noticeable both in sections and in whole preparations. That is, that the optic trunk leading to the regenerated appendage is much smaller in diameter than the one opposite. This fact suggests that probably only a part of the fibers of the optic nerve have persisted (Figs. 34, 38).

Large blood sinuses are present in the heteromorphic appendages. Aside from these no tissues are apparent except the hypodermis and the fibrous sheath which encloses the nerve bundles and the nerves themselves. In some instances muscles are found in the base of the appendage but these are probably remains of the base of the eye stump.

Material has been insufficient to make detailed observations upon the character of the masses of sensory cells found in the ganglion-like groups throughout the appendage. Sufficient observations have been made, however, to warrant the conclusion that they are concerned with the innervation of the hollow sensory hairs. In a few instances processes have been traced into the bases of the hairs which open by a wide mouth into the interior of the appendage (Figs. 78 and 79).

Herbst has considered the microscopic structure of these heteromorphic appendages in considerable detail and has examined a number of different stages. He describes the nerve cells as grouped into spindle-shaped ganglia with groups of nerve fibers extending from each end of the spindle-shaped masses, the distal bundle of strands being connected with the sensory hairs while the proximal bundle passes inward toward the brain. None of the stages examined by Herbst were younger than about six months, however, and consequently any structures that had developed would likely be much more definitely organized than in the appendages examined in this series of experiments.

Herbst considers that these ganglion-like groups of cells have developed from the hypodermis and that in the earlier stages they have no direct connection with the brain. In later stages, however, he describes the proximal bundles of the several ganglia as uniting to pass inward to the brain. But in most cases at least he considers that there is no union with the old optic nerve and consequently that the connection of the appendage with the brain is secondary. He mentions the similarity between these epithelial sense cells and those found in the first antenna, homologizing the sensory hairs which are found on the appendage with the olfactory setæ found upon the first antenna. Finally he comes to the conclusion that both in form and structure the heteromorphic append-

age shows that it should be regarded as a rudimentary first antenna. The structure of the heteromorphic appendage regenerated by the hermit crab agrees in certain respects with the observations of Herbst upon the structure of the heteromorphic appendages regenerated by other forms. In other respects the observations made upon hermit crabs are not sufficiently extensive to have any particular weight either way. The most significant difference, however, between these observations and Herbst's is in regard to the relation of the old optic nerve stump to the nerve bundles extending through the appendage. In the heteromorphic appendages regenerated by the hermit crabs there are several cases in which there can be no doubt as to the continuity of the optic nerve with the nerves in the appendage (Figs. 28, 34, 38). Further, these are found in stages younger than any spoken of by Herbst.

The continuity of the optic nerve stump and the nerve trunk of the heteromorphic appendage will be considered in all of its aspects in the general consideration of the problem of such heteromorphic regeneration.

E GENERAL CONSIDERATION OF REGENERATION FOLLOWING REMOVAL OF ENTIRE EYE

It has been seen in some cases that hermit crabs and *Crangon* regenerate an antenna-like appendage in place of an eye. On the other hand, the species of *Palæmonetes* used in this series of experiments has never shown any indication of such regeneration. In view of this, the question which naturally arises is why do we not find antenna-like appendages growing from the eye stumps of *Palæmonetes vulgaris*, when hermit crabs and *Crangon* kept under the same condition do regenerate these structures, and when the phenomenon is of pretty general occurrence among the Decapods. Herbst has observed the development of an antenna-like appendage from the eye stumps of a number of stalked-eyed Crustacea belonging to different families. He has even secured a few cases of this heteromorphosis in another species of *Palæmonetes* (*P. varians*). Morgan ('99) was the first to make the observation for

hermit crabs and a like phenomenon has been noted for three species of crayfish, *Cambarus virilis* and *C. gracilis* (Steele '04) and the blind crayfish, *C. Pellucidus testii* (Zeleny '06).

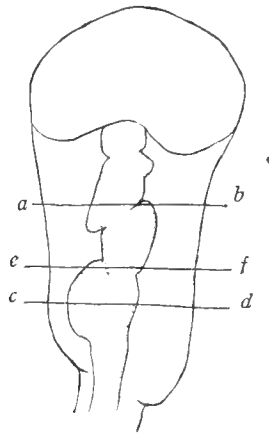
Widespread as the phenomenon appears to be, however, no satisfactory explanation of the cause of such heteromorphic regeneration has yet been suggested. Also an explanation of the negative cases, that is, where no particular regeneration takes place, is equally wanting. In the explanation of any phenomenon it is essential that negative cases be taken into account before any general conclusions are drawn. As has been pointed out above, even among the hermit crabs where the heteromorphic appendages appeared most frequently, in by far the majority of cases no regeneration took place. There was in these experiments a single series of hermit crabs in which nine out of fourteen individuals regenerated a heteromorphic appendage. In the light of this, we should perhaps be safe in concluding that for hermit crabs failure to regenerate may often be due to external conditions. But this would still explain nothing for *Crangon* and *Palæmonetes*.

All of the *Crangon* experimented upon belonged to the same series and were kept as nearly as possible under precisely the same conditions. Yet but one out of the original twenty-two developed an antenna-like appendage notwithstanding there were fourteen others that lived as long or longer and moulted as frequently. In so far as it was possible to determine the question, the physiological activity of the fourteen that showed no regeneration was equal to that of the one individual that did regenerate the appendage.

Extensive series of *Palæmonetes* were operated upon at the same time with the hermit crabs, and were kept under similar conditions. Yet, as has been seen not one regenerated the antenna-like appendage. From this it appears evident that, whatever variations in results may be accounted for by differences in external conditions, the primary answer to the question must be sought elsewhere.

It may be objected that in operating upon the eye the entire optic ganglion was not always removed. This, however, could not be offered as an objection in every case. In all three of the forms there were many instances in which not a vestige of the optic ganglion remained, and yet no regeneration resulted. Besides

there is evidence that in some cases hermit crabs regenerate an antenna-like appendage when part of the ganglion has been left in the eye stalk. We have then the following conditions for hermit crabs at least. First, when the cut comes at a level which leaves as much as two sections of the optic ganglion intact an eye may regenerate (*a-b*, text Fig. 2). Second, when the cut is made at the base or slightly above the base of the eye stalk (*c-d*, text Fig. 2) so that little or none of the optic ganglion remains the regeneration of an antenna-like appendage is possible. Lastly, if the eye is removed at a level intermediate between *a-b* and *c-d* (text Fig. 2) no regeneration follows.



Text Fig. 2 The line *a-b* represents approximately the level from which a hermit crab may regenerate an eye. From the level of the line *c-d* or below it a heteromorphic appendage may regenerate. No regeneration takes place from intermediate level, e.g., from the level *e-f*.

It is possible, perhaps even probable, that the character of the hypodermis differs more or less at these different levels. It is even conceivable that the hypodermis should be capable of one sort of regeneration at the level *a-b* or above it, and of another sort at the level *c-d* or below it; but there is certainly no apparent reason why no regeneration whatever should take place if the eye is removed at a plane intermediate between these two levels. So far as careful microscopic examination can determine there is no difference in the hypodermal cells underlying the cuticle proximal

to the basement membrane. Whatever differences in character may exist between the hypodermal cells over different regions of the eye, the results of this whole series of experiments suggest the inference that presence or absence of a maximum amount of the optic ganglion is a controlling factor in determining the character of the regeneration. The fact that no regeneration takes place from levels intermediate between *a-b* and *c-d* is in itself evidence that internal conditions are different at these intermediate levels than from a higher or a lower level. So far as the optic ganglion may be a controlling factor the difference in conditions may be due either to a difference in the character of the ganglion cells or to the reduced ganglionic mass. From the structure of the optic ganglion (Parker '90 and Kenyon '97) it is probable that not until the lower level *c-d* has been reached have the peripheral terminations of the optic nerve fibers been seriously interfered with. Both Parker and Kenyon mention the fact that a part of the optic nerve fibers have their cellular origins located in the brain. The fact that this heteromorphic appendage never regenerates except from this lower level suggests that there may be a causal connection between the regeneration of the heteromorphic appendage and the destruction of the distal terminations of the optic nerve fibers. With their peripheral terminations destroyed there might probably be a tendency on the part of the optic nerve fibers to grow outward and form new terminations. Since their natural terminations, the cells of the optic ganglion have been destroyed it seems probable that the fibers of the optic nerve stump would behave like those in a nerve stump of an ordinary appendage, *e.g.*, a leg or antenna. This in itself might have a tendency to induce any new tissue that regenerated to differentiate into the form of some sort of appendage.

That this heteromorphic appendage should be antenna-like in form seems probable for two reasons. First, it is the natural tendency of all Arthropod structures to divide into segments. Second, the simplest form of joint found in any appendage is in the antenna. Further, this appendage, although antenna-like, shows a much greater variety in form than any ordinary regenerated appendage and the joints formed are often irregular and

incomplete. This fact suggests that the regeneration was not influenced by a fixed set of internal conditions. In the usual cases of regeneration and embryonic development, whatever the determining factor or factors may be, it is recognized that we may expect certain structures to appear in connection with a given set of external and internal conditions.

In the development of this heteromorphic appendage, however, conditions seem more variable. As a consequence it shows considerable variety of form. In some cases the appendage is but little more than a slender horn-like projection, in other cases the appendage may be curved inward toward the median line, project forward at the angle of the eye or curve backward until the free end touches the margin of the head. (Compare Figs. 23, 38 and 41.) Again from the very first moult the appendage may appear as a single flagellum-like structure or as a pair. None of the hermit crabs, however, have regenerated a heteromorphic appendage composed of two flagellum-like parts. But in my previous observations upon crayfish (*loc. cit.*) two or three instances were noted in which the appendage appeared double at the time of the first moult. Herbst has also noted what he regards as an endopodite and exopodite in several instances. The appearance of the single structure in some cases and the double one in some others can perhaps be explained by the supposition that the nerve fibers become separated into two masses in some instances and remain as a single trunk in others. Miss Reed (*loc. cit.*) found that when the stump of the leg of a crayfish or hermit crab was split longitudinally in some instances two legs were regenerated from a single stump and in other cases only one. Sections of such legs showed that the end of the nerve stump had been split in the cases in which two legs regenerated and that the nerve stump had not been split when only one leg was regenerated. A similar result might follow in the development of the heteromorphic appendage if the nerve trunk became separated into two bundles by the interposition of another sort of tissue.

An explanation of the antenna-like form of the heteromorphic appendage having been suggested, attention should now be directed toward an explanation of its inner structure, which is also

found to be antenna-like. That its inner structure should be antenna-like might be expected since its innervation is associated with a region of the central nervous system that is particularly concerned with the innervation of the special sense organs, and since its outward form is antenna-like it is rather to be expected that the inner structure would also conform more or less to the antenna type.

It seems evident that the ganglionic groups of sense cells which are found in the heteromorphic appendage, belong to the general peripheral nervous system found so widely distributed among the different Arthropods. The groups of cells and the associated sensory hairs are equivalent to the "Hautsinnesorgane" of vom Rath ('94). Ost (*loc. cit.*), however, does not regard these sense cells as true ganglion cells, as Herbst does. In the regenerating antenna of *Oniscus*, Ost finds the nerve fibers regenerating from the central stump and the groups of sense cells differentiating from the hypodermis. The regenerating nerve fibers come from the end of the nerve stump, extend to the periphery and intermingle with the sense cells. Bethe ('96) considers that the peripheral nervous system of Arthropods differs both in function and origin from the central nervous system. Holmgren ('95) regards it as a sort of sympathetic system.

That cutting the peripheral terminations of the optic nerve may induce the regeneration of a heteromorphic appendage seems to receive some support from the results obtained by Zeleny upon the blind crayfish. Although reduced in size the optic ganglion is still present in the rudimentary eyes of blind crayfish. On the other hand the ommatidial structures are entirely wanting. So long as the vestigial eye remains undisturbed there seems to be no tendency toward the development of an antenna-like organ. But when the optic ganglion is removed a heteromorphic appendage appears. Such appendages are apparently functional as sense organs and Zeleny concludes that in the blind crayfish a non-functional organ has been replaced by a functional one.

The suggested explanation for the outgrowth of the heteromorphic appendage also carries with it an implied explanation of the non-appearance of a heteromorphic structure in place of a

somatic appendage. The nerve trunk of an appendage is associated with ganglion cells only at its central end, not with ganglion cells at its peripheral end, as distinguished from the optic nerve in its relation to the optic ganglion, consequently in removing an appendage no parts have been removed that would not be likely to again regenerate in a similar manner. While in animals as highly specialized as the hermit crabs we do not find the ganglion parts of the nervous system regenerating.

For the negative cases that appear after the entire optic ganglion has been removed, it is evident that no real explanation can be offered until a more adequate understanding of the process of growth and development has been reached. Although we may fully recognize the fact that great differences exist in the physiological activity of the various individuals and that the external conditions are subject to numerous variations, these facts alone will not account for the great number of negative cases which result. In addition to these it seems necessary to recognize an individual variation in the quality of the tissues. Nothing short of some specific inherent individual difference seems sufficient account for the fact that only an occasional hermit crab regenerates a heteromorphic appendage. The ability to regenerate a heteromorphic appendage in place of an eye which appears as an individual variation in hermit crabs and *Crangon* and other genera seems to be entirely wanting in at least one species of *Pakemonetes*. Or if not entirely wanting it appears so rarely that even after a great number of experiments and observations it is apparently absent.

In summing up the foregoing discussion it is apparent that a weight of responsibility has been placed upon the nervous system. Numerous observations, however, have left no doubt that the nervous system does exercise an important physiological influence upon the other tissues of the body, both in ordinary growth phenomena and in regeneration. Child ('04) observed in operating upon *Leptoplana* that if more than half of the cerebral ganglion was removed a new head did not regenerate. This was true regardless of the plane in which the cut was made, a fact which seems to indicate that the mass of nervous material is an important factor in the case of *Leptoplana* at least. Wilson ('03)

discovered that after the larger chela of *Alpheus* had been removed, cutting the nerve in the smaller one prevented it from growing into the form of the larger one when, however, the large chela had been removed and the nerve in the small one left intact, the small chela developed into the form of the large one. It has been noted above that Miss Reed found she could obtain the regeneration of the double chelæ. The experiments of Schaper ('98), Harrison ('03), Barfurth ('01), Goldstein ('04) and others have shown that the early stages of embryonic development and of regeneration are apparently independent of the nervous system. But the same experiments have also shown that the later stages of growth and differentiation are very largely influenced by the part of the nervous system which normally innervates the regenerating or developing parts; other instances might be mentioned but a sufficient number have been given to convince one that in very many instances there is an important connection between the part of the nervous system immediately concerned and the regeneration of the other tissues and structures.

VII REGENERATION AFTER SPLITTING THE EYE LONGITUDINALLY

Several series of *Palæmonetes* were operated upon by having the eye split longitudinally (Table I). Although in the regeneration of any part of the eye the new tissue is derived from the hypodermis the results obtained from the experiment of splitting the eye seem to indicate that injury to the optic ganglion is of great importance. In many cases at least splitting the eye could not have resulted in serious injury to the hypodermis yet in no case did regeneration follow if the optic ganglion had been injured. Whether or not regeneration followed the operation apparently depended upon the depth of the split. If the split extended through the ommatidial portion only and the optic ganglion remained uninjured, the ommatidial portion degenerated and new ommatidia were in some cases regenerated. On the other hand, if the split extended into the optic ganglion the whole ommatidial portion and the whole or part of the optic ganglion degenerated.

In some cases not even a vestige of the eye remained; in others, stumps of considerable length persisted. But in no cases where the split extended into the optic ganglion was there any sign of regeneration.

Figs. 16 and 22 represent instances in one of which an eye regenerated and in the other there were no signs of a regenerating eye. The specimen from which Fig. 16 was taken lived sixty-five days after the eye was split. The regenerated eye is about six-sevenths of the length of the normal eye. Sections show that new ommatidia have regenerated. The eye is not altogether normal in structure, however. The eye stump shown in Fig. 22 was taken from an individual that lived seventeen days after the operation. Apparently the entire optic ganglion has degenerated. There are no definite indications of regeneration. The stump of the optic nerve tapers to a point, perhaps indicating that degeneration is still incomplete. The stump is little more than one-third the length of normal eye.

No additional facts of importance were gained from the experiment of splitting the eye. These results obtained serve chiefly as additional proof that an injury to any part of the eye is followed by widespread degeneration of the tissues and that in the case of *Palæmonetes*, after an injury to the optic ganglion usually no regeneration takes place.

SUMMARY

In summing up the results of the experiments discussed in this paper the following points are to be noted:

- 1 The death of the animal which so frequently follows immediately upon the operation is perhaps due rather to its effect upon the nervous system than to loss of blood.

- 2 The healing of the wound takes place by the formation of a provisional crust over the cut surface and later by the development of a new cuticle beneath this crust.

- a* The crust is formed of hypodermal cells and a chitinous secretion. Intermingled with this are blood cells and the cells of the injured tissues. From two to three days are required for the formation of the crust.

b The new cuticle is secreted before a continuous hypodermis has formed over the wound. It is continuous with the inner layers of the cuticle over the eye stump.

3 New hypodermal cells over the ommatidial region may arise in two ways; either by the transformation *in situ* of corneal hypodermal cells into less specialized, actively multiplying hypodermal cells, or by the proliferation of new hypodermal cells inward from the edges of the cut.

4 Any injury to the eye is always accompanied by extensive degeneration of the remaining tissues. Sometimes the entire eye suffers destruction.

5 The rate of regeneration is considerably affected by the rate of disintegration and the removal of injured parts.

6 Active regeneration may be in progress at the periphery while deeper below the surface the injured structures are not yet removed.

7 In the regeneration of an eye all of the new structures arise from the hypodermis.

8 Multiplication of cells takes place by amitotic divisions.

9 The cells for the retinulae are the first to differentiate from the hypodermis. Their differentiation may begin before a continuous hypodermis has developed.

10 The reticular nuclei move inward from the periphery, elongate and divide along their radial axes, and extend proximal processes through the basement membranes to the optic ganglion. Thereby nervous connections are established in the regenerating region.

11 Not until after the reticular processes have extended into the optic ganglion is the differentiation of cones established. The cones differentiate from the periphery inward.

12 The rhabdom is developed from the inner ends of the reticular cells and is at first present as a slender homogeneous rod of uniform diameter, which extends from the inner end of the cones to the basement membrane. The spindle shaped enlargement of the rhabdom does not appear until after all the other parts of the ommatidium have been differentiated.

13 The hypodermis does not become a true corneal hypodermis

and secrete corneal facets until after all of the other ommatidial structures have been differentiated. Corneal facets are never apparent until after more than one moult has taken place.

14 Ommatidia do not differentiate at a uniform rate in all parts of the regenerating eye.

15 In *Palæmonetes* regeneration of perfect ommatidia does not take place if the optic ganglion has been injured. Hermit crabs may regenerate a perfect eye after removal of as much as half the optic ganglion. Crangon regenerates an eye very slowly, even when the optic ganglion is uninjured, but there are evidences that ommatidia may differentiate after a part of the optic ganglion has been removed.

16 The rate of regeneration is quite variable in all the species experimented upon, but both hermit crabs and *Palæmonetes* however may regenerate ommatidia within thirty-five to forty-five days.

17 Splitting the eye of *Palæmonetes* is not followed by regeneration if the split extends into the optic ganglion.

18 In the breaking down of the injured ommatidia the pigment secreting cells become widely scattered, and the old pigment persists for a long time. Frequent cases of abnormal development of pigment also occur. There are evidences which indicate that this abnormality is due to the pathological development of the broken down retinulæ.

19 After removal of all or nearly all of the optic ganglion, hermit crabs may regenerate a heteromorphic appendage in place of the excised eye. There is, however, apparently a level from which neither an eye nor an antenna-like appendage will regenerate.

20 The nerve-trunk of the heteromorphic appendage forms a continuous structure with the stump of the optic nerve.

21 Removal of the entire eye of Crangon may also be followed by the regeneration of an antenna-like appendage.

22 In no case was there evidence that *Palæmonetes vulgaris* possessed the ability to regenerate a heteromorphic appendage after the removal of the entire eye.

23 The results of this entire series of experiments points to the

following conclusion. The regeneration which takes place from any level is largely influenced by the presence or absence of the whole or a part of the optic ganglion.

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EXPLANATION OF PLATES

Outlines of all figures were drawn with the aid of a camera. In all of the detailed figures the nuclei were also drawn in with the camera. The magnification is given with the explanation of each figure. As far as possible the figures illustrating the different phases of the subject are numbered according to the number of days the experiment covered.

Reference letters used

<i>a.</i>	anterior.	<i>n.c.t.</i>	new cuticle.
<i>a.cp</i>	anterior border of carapace.	<i>n.e.</i>	normal eye.
<i>a.p.c.</i>	abnormal pigment cells.	<i>n.</i>	nucleus.
<i>bm.</i>	basement membrane.	<i>n.tr.</i>	nerve trunk.
<i>br.</i>	brain.	<i>o.ct.</i>	old cuticle.
<i>br.sh.</i>	brain sheath.	<i>c.m.</i>	old muscle.
<i>c.c.</i>	crystalline cones.	<i>o.p.n.</i>	optic nerve.
<i>c.hy.</i>	corneal hypodermis.	<i>o.s.p.</i>	optic squame.
<i>c.p.</i>	coagulated plasma.	<i>p.</i>	posterior.
<i>cr.</i>	crust.	<i>pt.</i>	pigment.
<i>ct.</i>	cuticle.	<i>pt.cs.</i>	pigment cysts.
<i>e.s.</i>	eye stump.	<i>ret.</i>	retinulae.
<i>cf.</i>	corneal facet.	<i>ret.n.</i>	retinular nuclei.
<i>gl.</i>	ganglion.	<i>rh.</i>	rhabdom.
<i>het.</i>	heteromorphic appendage.	<i>r.t.</i>	regenerated tissue.
<i>hy.</i>	hypodermis.	<i>seg.</i>	segments.
<i>hy.tr.</i>	transformed hypodermis.	<i>s.ct.</i>	sub cuticle.
<i>m.</i>	muscle.	<i>sn.</i>	sensory hairs.

PLATE I

Fig. 1 *Palæmonetes*, seven days. One moult seven days after operation. *a*, Dorsal view, and *b*, ventral view. Most of ommatidia removed from ventral side. Pigmented portion appears disorganized. Injured eye measures about four-fifths length of normal eye. $\times 45$.

Fig. 2 Young *Palæmonetes*, seven days. One moult seven days after operation. Dorsal view. Eye operated upon by thrusting needle into top of ommatidial portion. Nearly half of the ommatidia destroyed. Injured eye measures about three-fourths length of normal eye. $\times 45$.

Fig. 3 *Palæmonetes*, ten days. One moult seven days after operation. Ventral view. Part of ventral ommatidial portion removed. Pigment irregularly scattered throughout ommatidial region. Very few uninjured ommatidia remains. $\times 45$.

Fig. 4 *Palæmonetes* eye, nineteen days. One moult eighteen days after the operation. Nearly whole ommatidial portion was removed. Pigment patches remains of old ommatidia. New tissues can be seen arranged in strands on interior edge. $\times 45$.

Fig. 5 Hermit crab, twenty-five days. Regenerated eye, one moult twenty-four days after operation. At least one section of optic ganglion removed. Regenerated eye five-eighths length of normal eye. $\times 45$.

Fig. 6 *Palæmonetes*, thirty days. First moult seven days after operation; second moult twenty-one days later. Ventral view. Whole ommatidial region destroyed. Upper part of regenerated tissue perfectly transparent. Irregular patches of old pigment remains seen in lower part of ommatidial region. Regenerating eye three-fourths length of normal eye. $\times 45$.

Fig. 7 *Crangon*, thirty-two days. First moult eighteen days after operation; second moult fourteen days later. Dorsal view. Operation removed upper ommatidial surface. Remains of old pigment apparent. Interior of eye shrunk away from cuticle. Injured eye four-fifths length of normal eye. $\times 60$.

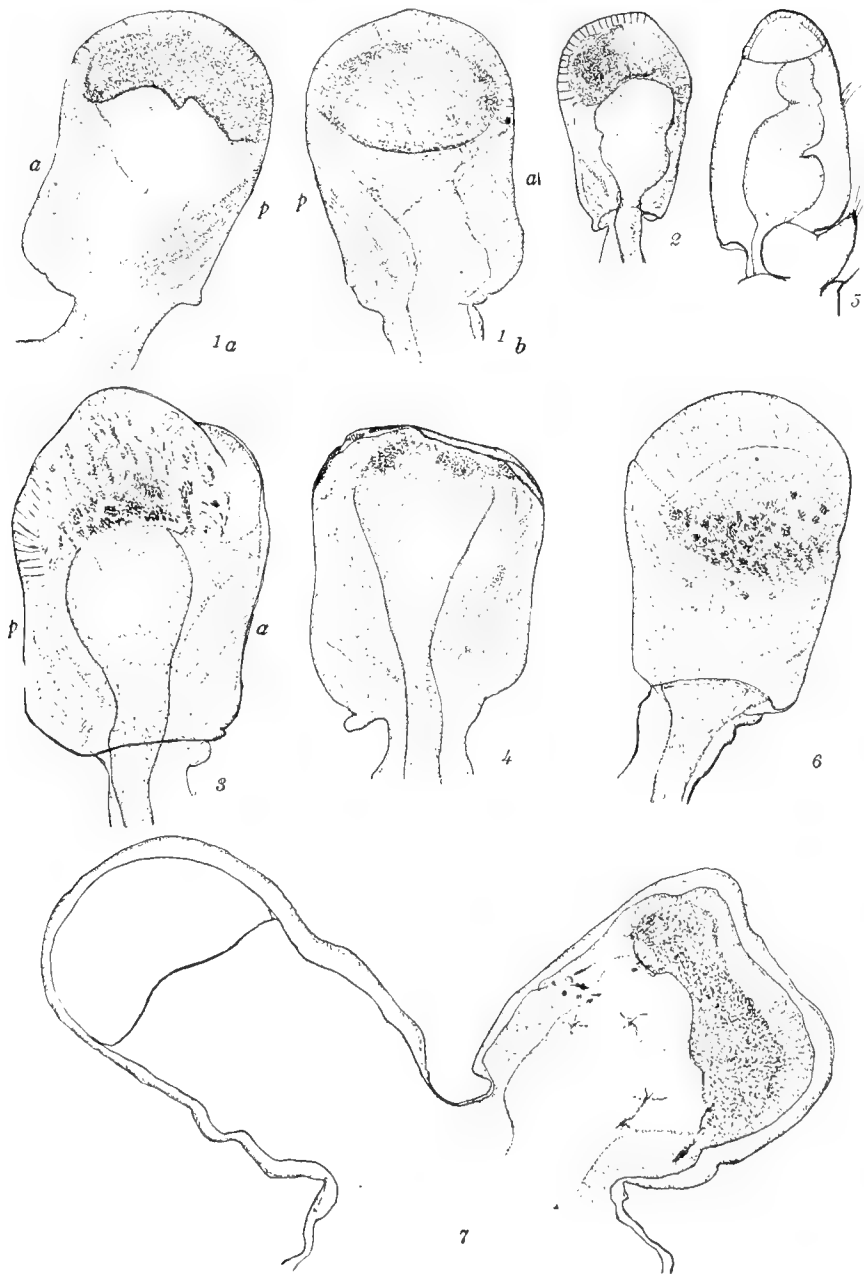


PLATE II

Fig. 8 Crangon, thirty-one days. One moult sixteen days after injury. Dorsal view. Operation removed small part of inner anterior ommatidial surface. Injured eye four-fifths length of normal. $\times 45$.

Fig. 9 Palæmonetes, thirty-three days. First moult fourteen days after operation; second moult nine days later. Dorsal view. Injury chiefly on posterior ventral edge. Injured eye four-fifths length of normal eye. $\times 35$.

Fig. 10 Palæmonetes, thirty-three days. First moult fourteen days after operation; second moult ten days later. Whole of ommatidial region destroyed. Regenerating eye four-fifths length of normal eye. $\times 35$.

Fig. 11 Palæmonetes, thirty-five days. First moult sixteen days after operation. Ventral view. Part of ommatidia removed from ventral side. Upper end of eye more pointed than usual. Pigment appears unevenly distributed. Regenerated eye seven-eighths length of normal eye. $\times 45$.

Fig. 12 Hermit crab, thirty-three days. First moult thirty-two days after operation. Dorsal view. Operation removed all of ommatidia and part of optic ganglion. Small, complete, new eye regenerated. New ommatidia shorter than normal. New eye two-thirds length of normal eye. $\times 45$.

Fig. 13 Hermit crab, thirty-eight days. First moult twelve days after the operation. Dorsal view. Ommatidial region and nearly half of the ganglion removed. Very small but perfect eye regenerated. Regenerated eye four-sevenths length of normal eye. $\times 45$.

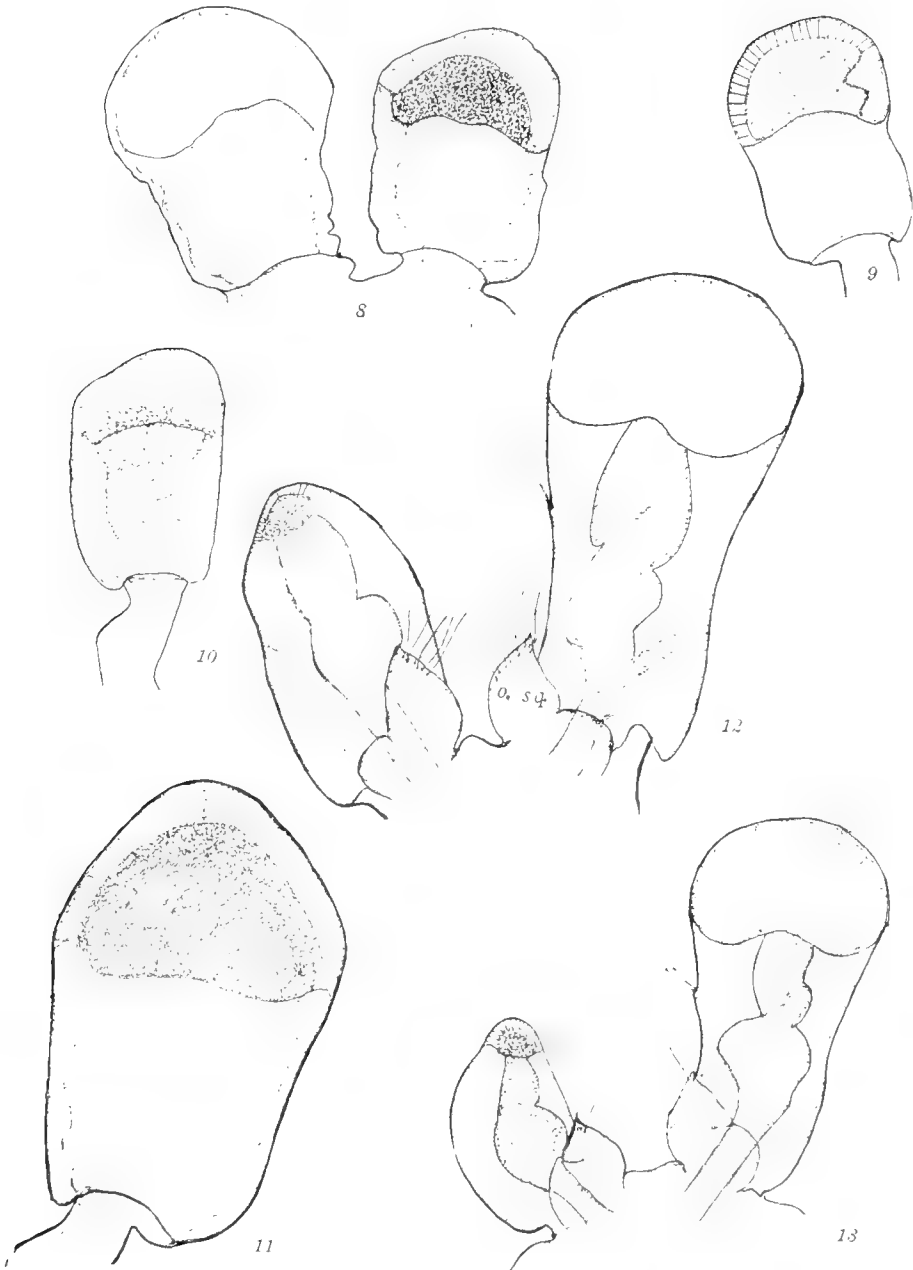


PLATE III

Fig. 14 Hermit crab, forty-one days. First moult forty-one days after operation. Dorsal view. Operation destroyed whole ommatidial portion and upper part of ganglion. Complete eye regenerated. Eye is about two-thirds length of normal eye. $\times 45$.

Fig. 15 Hermit crab, sixty-seven days. One moult forty-five days after operation. Whole ommatidial portion and upper part of ganglion destroyed. Regenerated eye fully differentiated. Regenerated eye two-thirds length of normal eye. $\times 45$.

Fig. 16 *Palæmonetes*, sixty-five days. Moulded six days after operation. Eye split. New ommatidia regenerated. Regenerated eye six-sevenths length of normal eye. $\times 45$.

Fig. 17 *Palæmonetes Viridis*, nine days. One moult nine days after operation. Shows irregular development of upper end of eye. Pigment scattered irregularly. $\times 45$.

Fig. 18 Ventral view of top of eye shown in Fig. 40. Regenerated material appears loose and reticular. $\times 90$.

Fig. 19 *Palæmonetes*, thirty days. Dorsal view. First moult eight days after operation; second moult fifteen days later. Ommatidial portion wholly destroyed. Upper part of eye transparent. Regenerated tissue forms loose reticulum. No external signs of differentiation of ommatidia. Injured eye seven-eighths length of normal eye. $\times 45$.

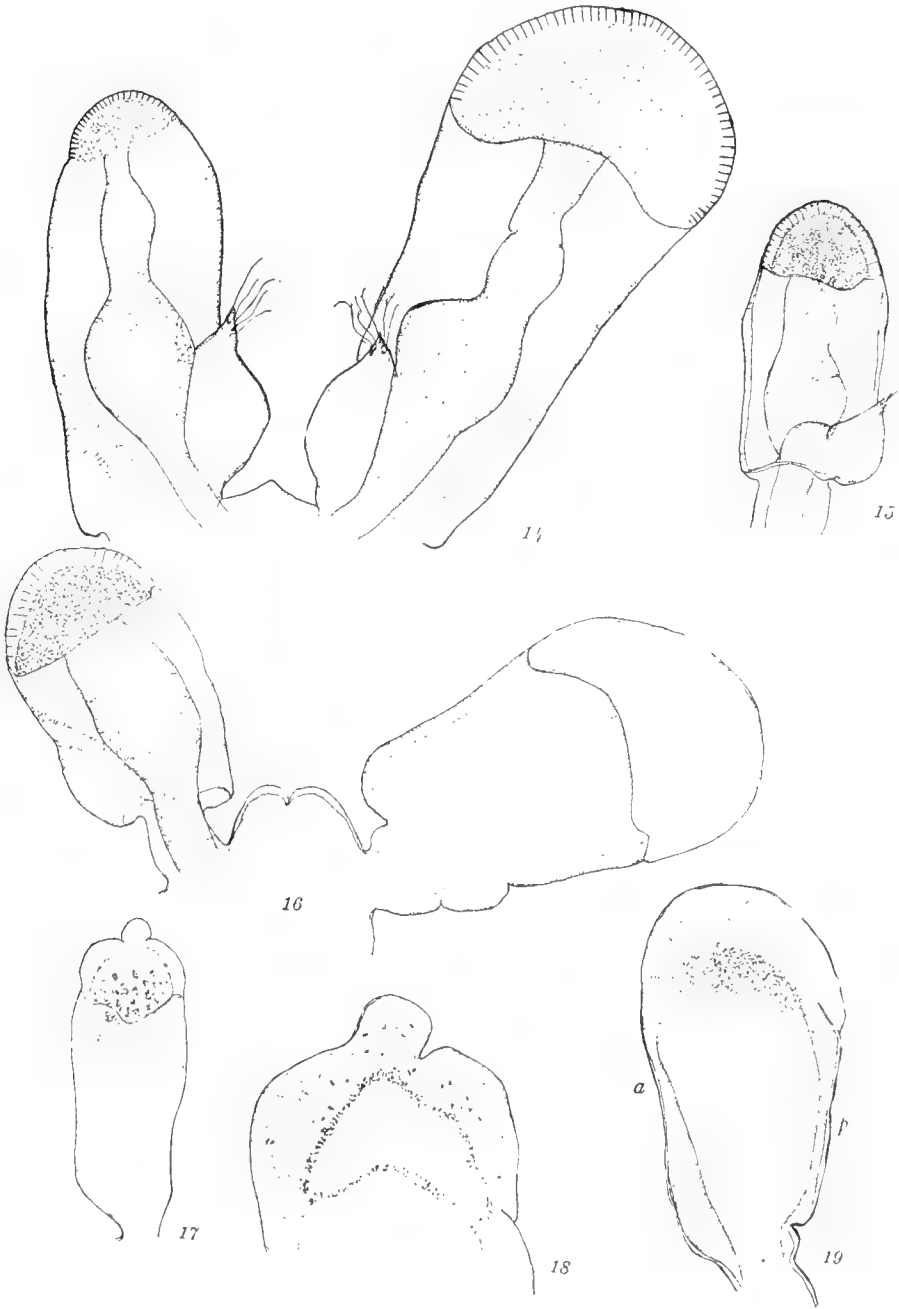


PLATE IV

Fig. 20 *Palæmonetes*, thirty-eight days. First moult ten days after operation; second moult fourteen days later. Ventral view. Entire ommatidial region destroyed. Dark band represents remains of old pigment. Regenerating eye five-eighths length of normal eye. $\times 45$.

Fig. 21 Hermit crab, sixty-seven days. Moulded twelve days after operation. Stump shows large part of ganglion remaining but no signs of regenerating eye. Loose shreds of new tissue developed distal to the stump. $\times 45$.

Fig. 22 *Palæmonetes*, seventeen days. Moulded seventeen days after operation. Eye split. Almost entire eye degenerated. Stump about one-third length of normal eye. $\times 90$.

Fig. 23 Hermit crab, twenty-one days. Regenerated heteromorphic appendage and base of normal eye. One moult twenty-one days after operation. Appendage segmented; curves inward toward the median line. $\times 90$.

Fig. 24 Eye stump, thirty-two days. First moult ten days after operation; second moult sixteen days later. Pigment patches near distal end of stump. Short stiff hairs on end of stump. Stump is two-fifths length of normal eye. $\times 45$.

Fig. 25 Hermit crab. Injured when found. One moult. Ventral view. Distal end of eye shows regenerating ommatidia at *o*. Abnormal pigment developed at *pt*. Abnormal protuberance on the inner edge. Injured eye three-fifths length of normal eye. $\times 45$.



PLATE V

Fig. 26 *Palæmonetes*, twenty-four days. First moult ten days after operation; second moult fourteen days later. Right eye removed at level of line *a-b*. Small bud of new tissue. *z*, Regenerated from stump. Cuticle removed from stump. $\times 45$.

Fig. 27 Stump shown in Fig. 26 more highly magnified. New tissue darkly shaded. $\times 125$.

Fig. 28 Hermit crab, twenty-four days. One moult twenty-two days after removal of eye. Heteromorphic appendage. Ventral view. Cuticle heavy, appendage irregularly segmented. Sensory hairs developing near tip. Nerve trunk visible beyond proximal half of segment. Nerve trunk small in diameter as compared with Fig. 30. Appendage three-fourths length of normal eye. $\times 90$.

Fig. 29 *Palæmonetes*, twenty-five days. One moult. Outline of normal eye and eye stump showing abnormal pigment. Pigment in a number of small masses on upper distal end of stump. $\times 75$.

Fig. 30 Hermit crab, twenty-two days. Moulded twenty-two days after operation. Heteromorphic appendage. Dorsal view. Show segments, sensory hairs and nerve trunk extending beyond proximal half of appendage. Appendage one-third length of normal eye. $\times 125$.

Fig. 31 Hermit crab, twenty-six days. One moult twenty-six days after operation. Dorsal view of normal eye and healed over stump. Stump three-eighths length of normal eye. $\times 45$.

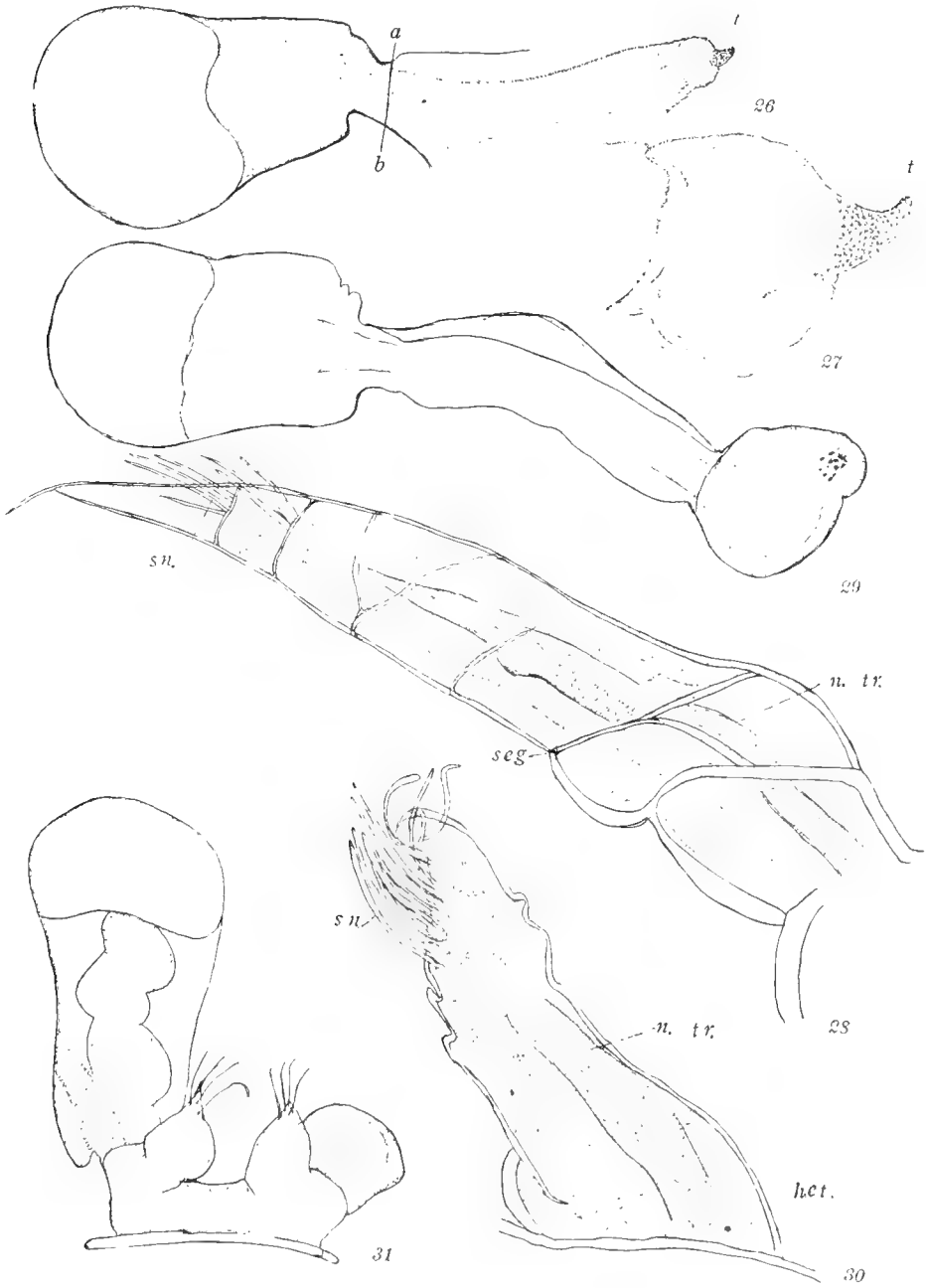


PLATE VI

Fig. 32 *Palæmonetes*, twenty-seven days. First moult sixteen days after operation; second moult ten days later. Outline of normal eye and eye stump showing abnormal pigment. Pigment appears as granular area on inner dorsal surface. Eye stump four-fifths length of normal eye. $\times 45$.

Fig. 33 *Palæmonetes*, twenty-seven days. First moult ten days after operation; second moult fourteen days later. Ventral view of stump and normal eye. End of optic nerve stump flush against the cuticle. Optic nerve reduced in size, two-sevenths lengths of normal eye. $\times 45$.

Fig. 34 Hermit crab, thirty-nine days. Moulded twenty-nine days after operation. Dorsal view of normal eye and heteromorphic appendage. Shows optic squame in connection with appendage. Optic nerve stump extends through proximal half of appendage. $\times 35$.

Fig. 35 Series of *Palæmonetes* eye stumps after removal of greater part of eye. *a*, Eye stump with small quantity of new tissue developed beyond end of optic nerve stump. Stump measures one-third length of normal eye. $\times 45$. *b*, Eye stump that shows no regeneration. Twenty-nine days. Moulded ten days after operation. One-fourth length of normal eye. $\times 45$. *c*, Eye stump showing an unusual development of new tissue. Moulded ten days after operation. Stump two-sevenths length of normal eye. $\times 45$.

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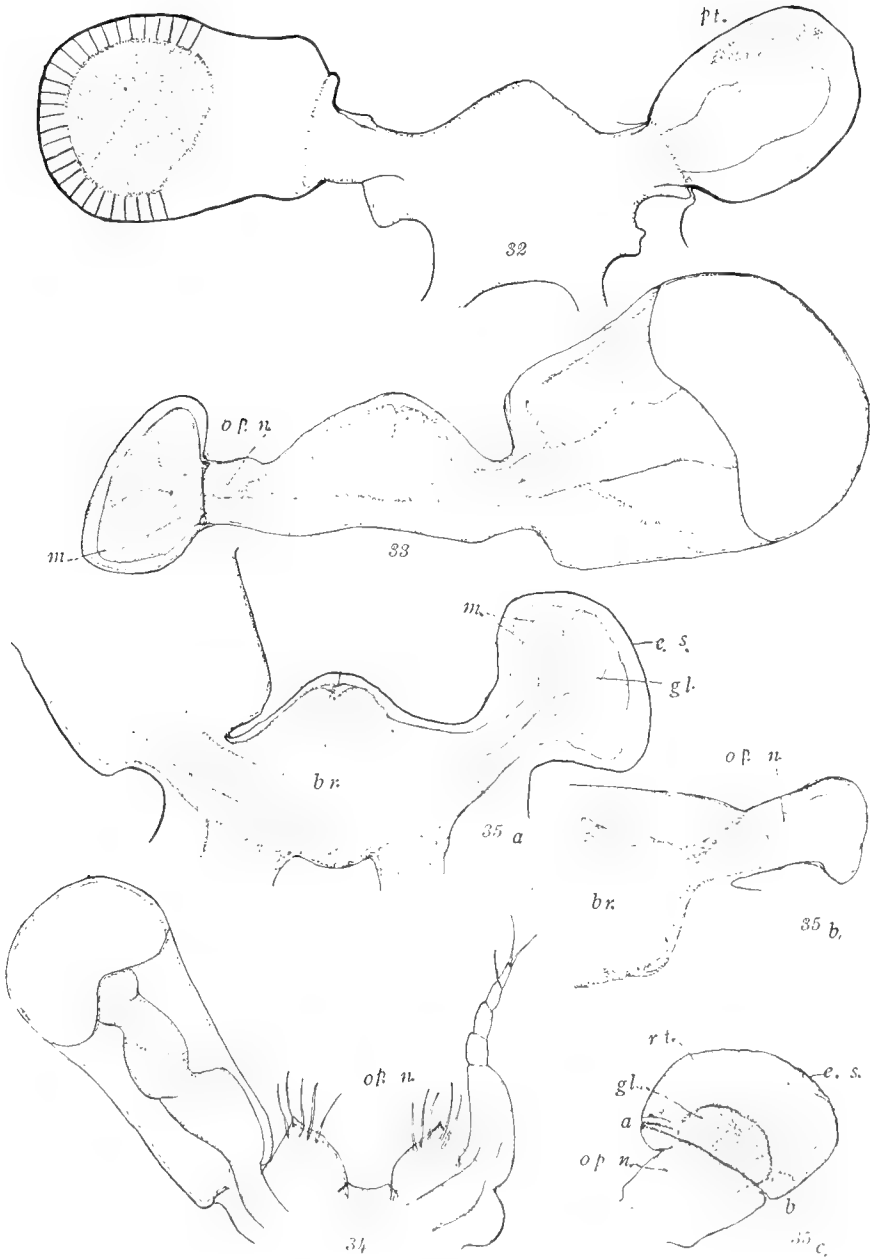


PLATE VII

Fig. 36 *a* and *b*, *Palæmonetes*, thirty days. One moult. Optical section of normal and regenerating eye. Ventral view. *a*, Shows regenerating eye; *b*, normal eye for comparison. Operation apparently removed eye near level of line *a-b* on normal eye. Regenerating eye shows considerable new tissue and pigment spot on ventral side. Heavy cuticle over end of stump. $\times 45$.

Fig. 37 Crangon eye stump, thirty-two days. First moult two days after operation; second moult nine days later; third moult sixteen days later. Stump one-third length of normal eye. No regeneration. $\times 45$.

Fig. 38 Crangon, thirty-two days. First moult four days after operation; second moult seventeen days later. *a*, Optical section of heteromorphic appendage and outline of normal eye. Dorsal view. $\times 45$. *b*, Ventral view of heteromorphic appendage more highly magnified. Shows six segments and sensory hairs developed on the inner distal edge. Nerve trunk apparent through greater part of length. Appendage measures four-fifths length of normal eye. $\times 90$.

Fig. 39 Hermit crab, thirty-two days. One moult thirty-two days after operation. Ventral view of stump showing no regeneration. Stump two-thirds length of normal eye. $\times 45$.

Fig. 40 Crangon eye stump, thirty-one days. First moult seven days after operation; second moult twelve days later. Stump measures one-third length of normal eye. $\times 45$.

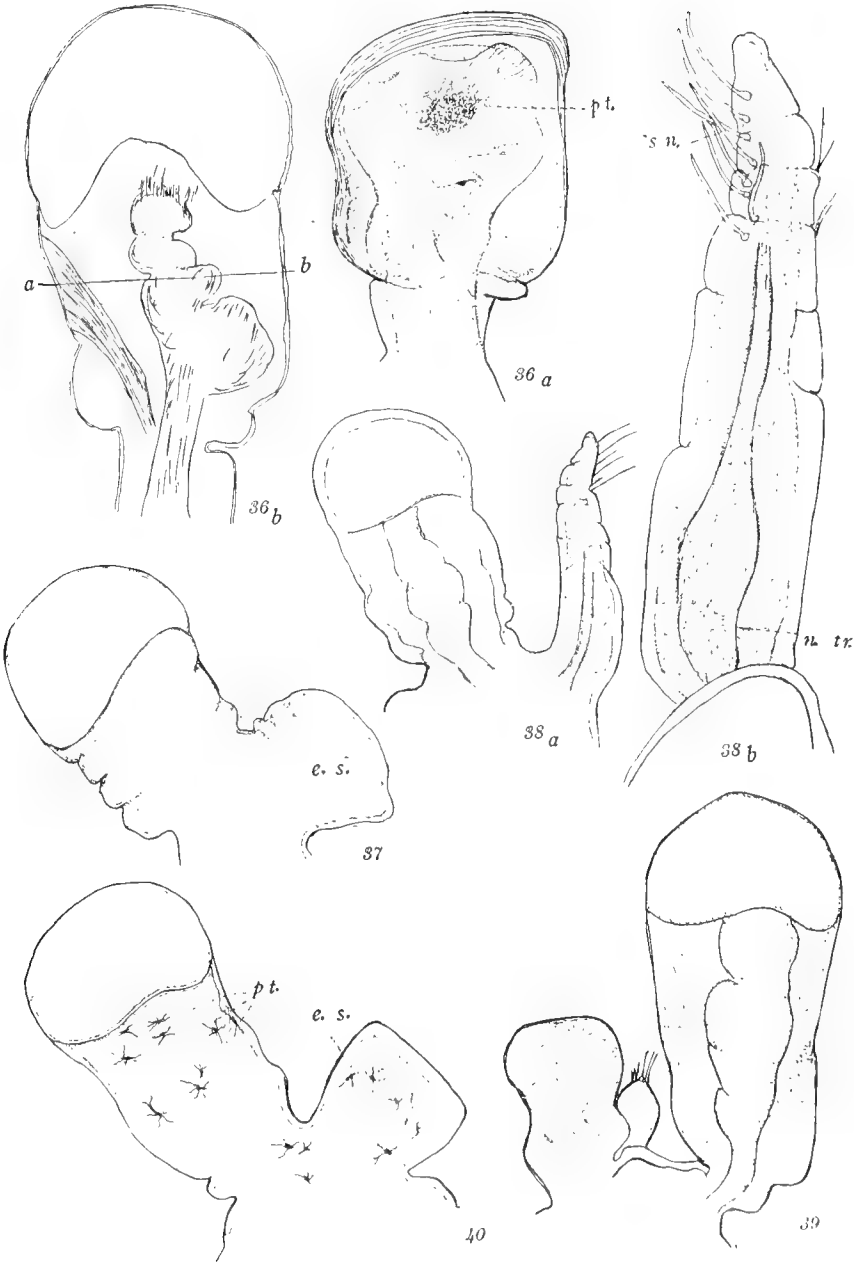


PLATE VIII

Fig. 41 Hermit crab, thirty-two days. One moult thirty-two days after operation. Dorsal view of normal eye and heteromorphic appendage. Appendage small and sharply curved backward. Shows several segments and a few sensory hairs. $\times 90$.

Fig. 42 Crangon eye stump, thirty-two days. First moult eight days after operation; second moult twelve days later. Cuticle folded and wrinkled. Short hairs on end of stump. Stump about one-half length of normal eye. No regeneration. $\times 45$.

Fig. 43 Palæmonetes, thirty-eight days. Two moults. Eye stump and outline of normal eye. Ventral view. Shows abnormal pigment spot. Eye stump one-half length of normal eye. $\times 45$.

Fig. 44 Palæmonetes, thirty-eight days. First moult sixteen days after operation; second moult eighteen days later. Eye stump showing abnormal pigment which appears as a single solid mass on upper anterior border of eye stump. Stump about two-thirds length of normal eye. $\times 45$.

Fig. 45 Hermit crab, thirty-nine days. One moult. Heteromorphic appendage. Dorsal view. Nerve trunk distinct in proximal part of appendage. Appendage three-fifths length of normal eye. $\times 90$.

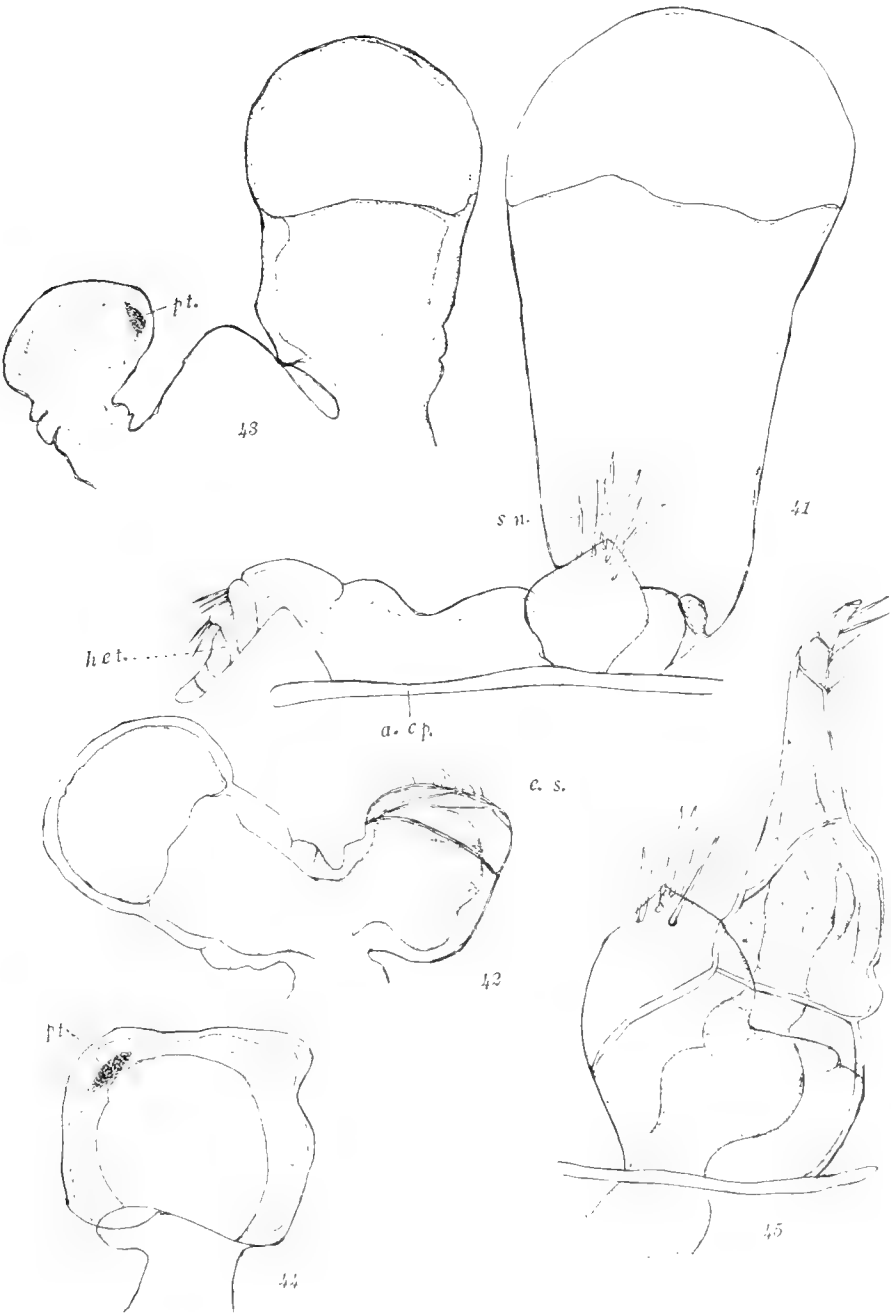


PLATE IX

Fig. 46 Semidiagrammatic section through the top of eye of *Cambarus virilis* sixty-two and one-half hours after removal of part of ommatidia. Shows relation of cuticle and protective crust (*cr.*) Shows broken down tissue excluded by crust also. Below crust is space from which inner tissues have shrunk. Space occupied by coagulated plasma. $\times 90$.

Fig. 47 Semidiagrammatic section through upper part of eye of *Cambarus gracilis* showing contiguity between regenerated and old cuticle. Also shows broken down tissues excluded by development of cuticle. Eye operated upon by tearing small hole in cornea with needle. $\times 450$.

Fig. 48 Section from eye shown in Fig. 9. Broad band of new cuticle developed. Few regenerated nuclei present. All the tissue shown below cuticle degenerating remains of old ommatidia. $\times 1350$.

Fig. 49 Section from eye shown in Fig. 3. Shows new cuticle with no hypodermal cells beneath it. Shows amitotically dividing nuclei. $\times 1350$.

Fig. 50 Part of section from eye shown in Fig. 3. Section from near edge injured area. Hypodermal nuclei much more numerous than in Fig. 51, which is taken from a section near center of injured area. $\times 600$.

Fig. 51 Section from eye shown in Fig. 3. New cuticle but no well defined hypodermis yet formed beneath cuticle. Granular masses and pigment patches are remains of degenerating ommatidia. $\times 430$.

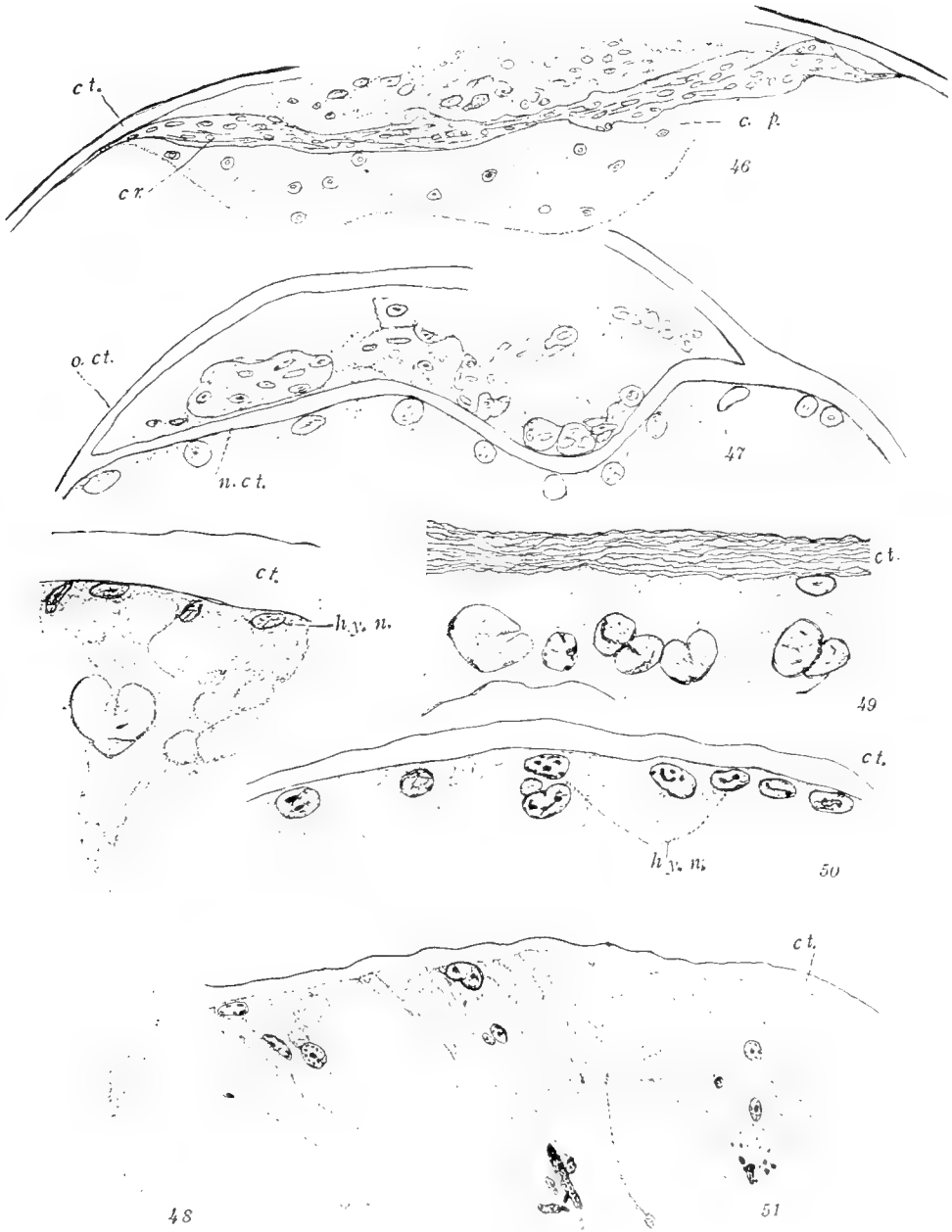


PLATE X

Fig. 52 *a, b, c, d* and *e* shows series of figures from an eye of *Palæmonetes* representing transformation of corneal hypodermal cells into active regenerating hypodermis. Shows normal corneal hypodermal cells together with corneal facets and tops of cones in *a* and *b*. Transformation of cells from resting corneal hypodermal cells to active regenerating cells in *c* and *d*. These form a continuous series. $\times 1350$.

Fig. 53 Section of eye of *Palæmonetes* from which part of the ommatidial region was removed. Shows new cuticle and reticular subcuticle. Transformed hypodermal cells in process of amitotic division. To the left a single cell which may be undergoing mitotic division. Experiment covered twenty-three days. Moulded twice. Section taken from same eye as series in Fig. 52. $\times 1350$.

Fig. 54 Sections from same eye as Figs. 52 and 53. This section shows cells separating from the hypodermis and also early stages of differentiation of retinulae. Outline of cuticle and subcuticle shown. $\times 660$.

Fig. 55 Section from eye shown in Fig. 10. Hypodermal cells differentiated. Amitosis taking place in these and the deeper lying cells. The deeper cells are regenerating reticular cells. $\times 1350$.

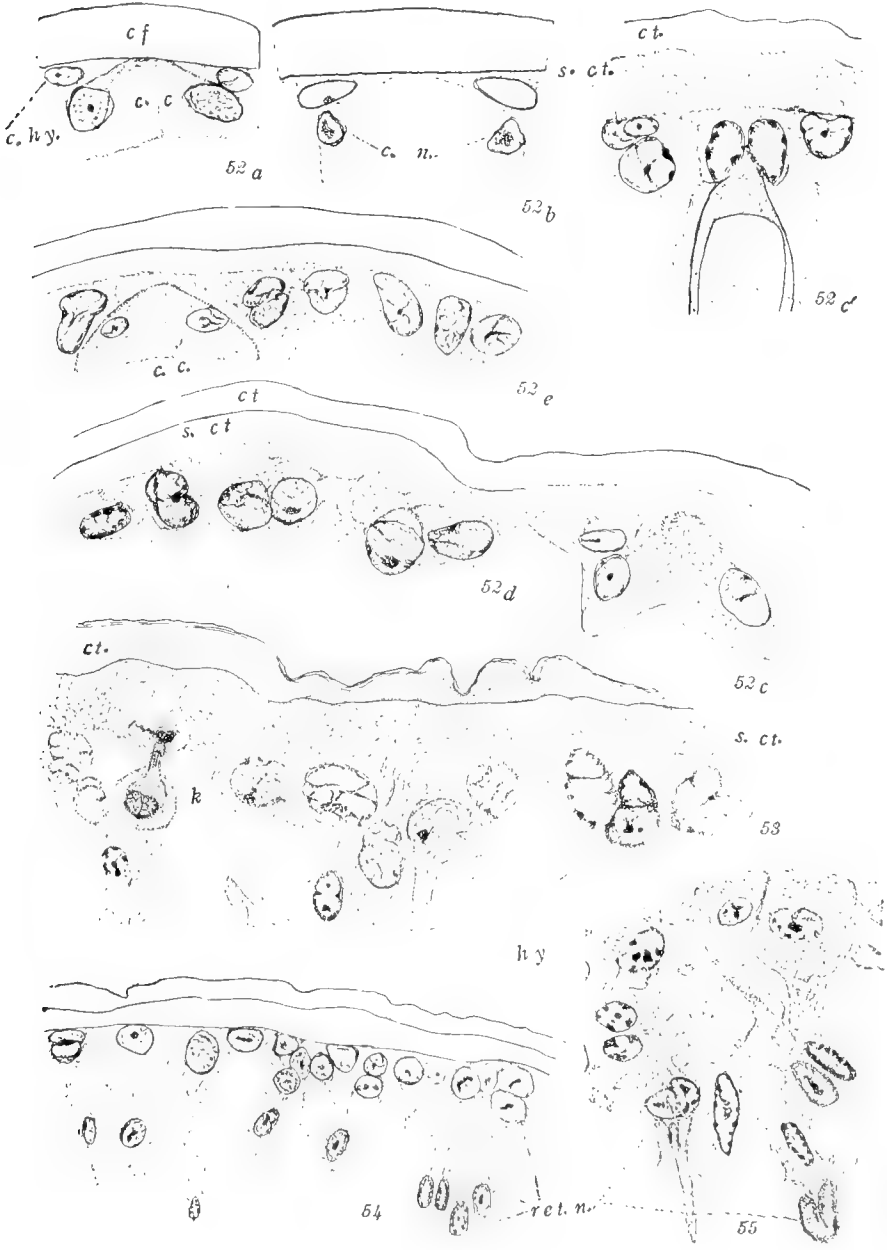


PLATE XI

Figs. 56-59 Taken from sections of regenerating eye shown in Fig. 36. Fig. 56 from section near dorsal surface. Regenerated tissue lies for the most part peripheral to the broken line *x-y*. Cuticle torn and inner tissues shrunk from it. Old tissues show parts of muscle bands and small groups of ganglion cells. Distal to muscle band new tissue seems differentiated into fibers. $\times 90$.

Fig. 57 Shows section deeper below surface than Fig. 35. Same features as in preceding figure. In addition a few small pigment masses. An increase in size of the nuclei in region from *b* to *c*. Cuticle not shown. $\times 90$.

Fig. 58 Represents upper part of tangential section near the ventral surface. Nuclei increased in size and number over those in preceding figure. Rudimentary ommatidial elements apparent in new tissue. Figure composed entirely of regenerated tissue except small group of ganglion cells. $\times 90$.

Fig. 59 Rudimentary ommatidia from the eye shown in Fig. 36. Sections oblique so that entire ommatidium cannot be recognized. Shows distal ends of cones, reticular nuclei and pigmented processes which appear to be retinulae. $\times 900$.

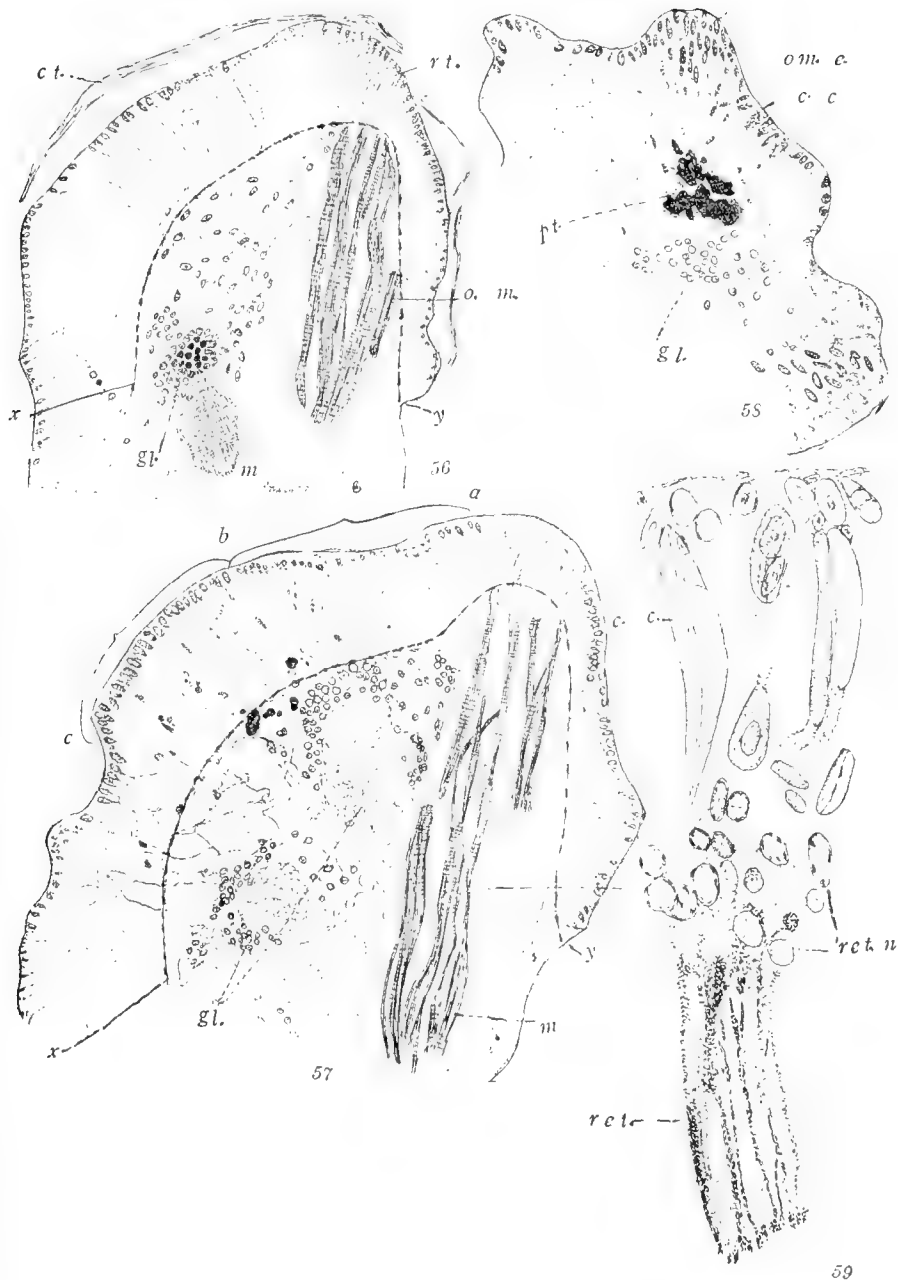


PLATE XII

Fig. 60 Group of retinulae from eye shown in Fig. 6. Proximal reticular processes are seen extending to basement membrane. Two of the processes can be traced through below the basement membrane. $\times 900$.

Fig. 61 Taken from section of eye shown in Fig. 16. Shows group of retinulae. Nuclei in outline and proximal processes shown. $\times 1350$.

Fig. 62 Group of retinulae from eye shown in Fig. 6. Shows proximal reticular processes penetrating basement membrane and twining among ganglion cells below. $\times 1350$.

Fig. 63 Taken from section of eye shown in Fig. 11. Shows early stage in differentiation of crystalline cones. Cone nuclei are being separated from hypodermal nuclei. Hypodermal nuclei are grouped in pairs. Delicate strands of cytoplasm extending inward from pairs of nuclei. $\times 1350$.

Fig. 64 Taken from section of eye shown in Fig. 11. Shows more advanced stage of cone differentiation than Fig. 29. Cell outlines becoming defined but hypodermal and cone cells not distinctly separated. Section somewhat oblique so that the four cone nuclei are visible. Distal reticular processes extending between the cones. Lower ends of cones not yet differentiated. $\times 900$.

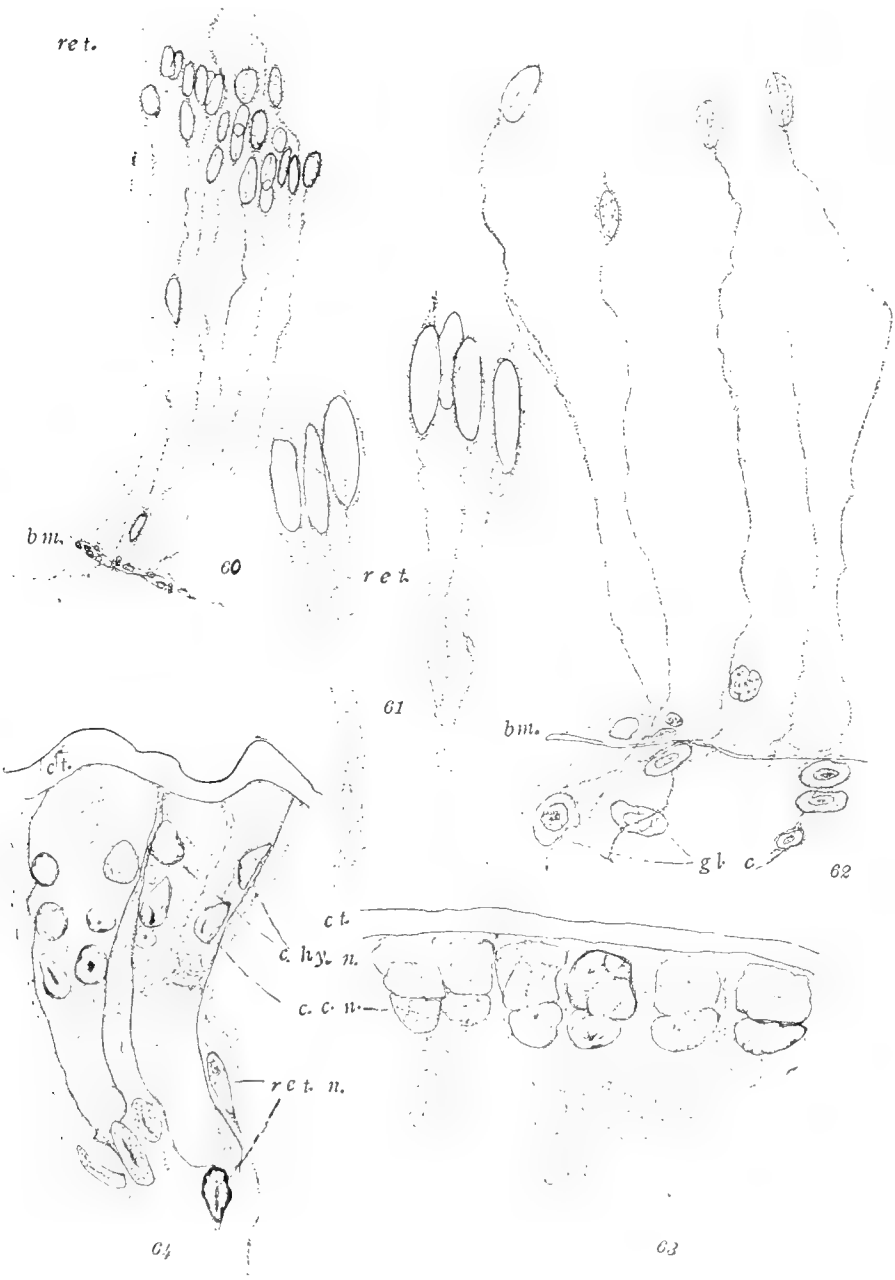


PLATE XIII

Fig. 65 Taken from section of eye shown in Fig. 11. Shows retinulae with their distal processes extending to hypodermis. Shows early pigment deposition in proximal processes. Hypodermal cells shown in outline. $\times 1350$.

Fig. 66 Taken from section of eye shown in Fig. 11. Ommatidia completely differentiated except spindle shaped enlargement of the rhabdom. Distal ends of cones not yet differentiated completely. Cone at left of figure cut obliquely. Retinulae not altogether normal in their distribution. $\times 1350$.

Fig. 67 Regenerated ommatidium from eye. Shown in Fig. 11. Rhabdom still not quite normal in appearance. $\times 1350$.

Fig. 68 Sections from an eye shown in Fig. 15. Shows differentiated hypodermis and retinular nuclei beginning to assume their definitive position. Hypodermis and retinulae both show dividing nuclei. $\times 1350$.

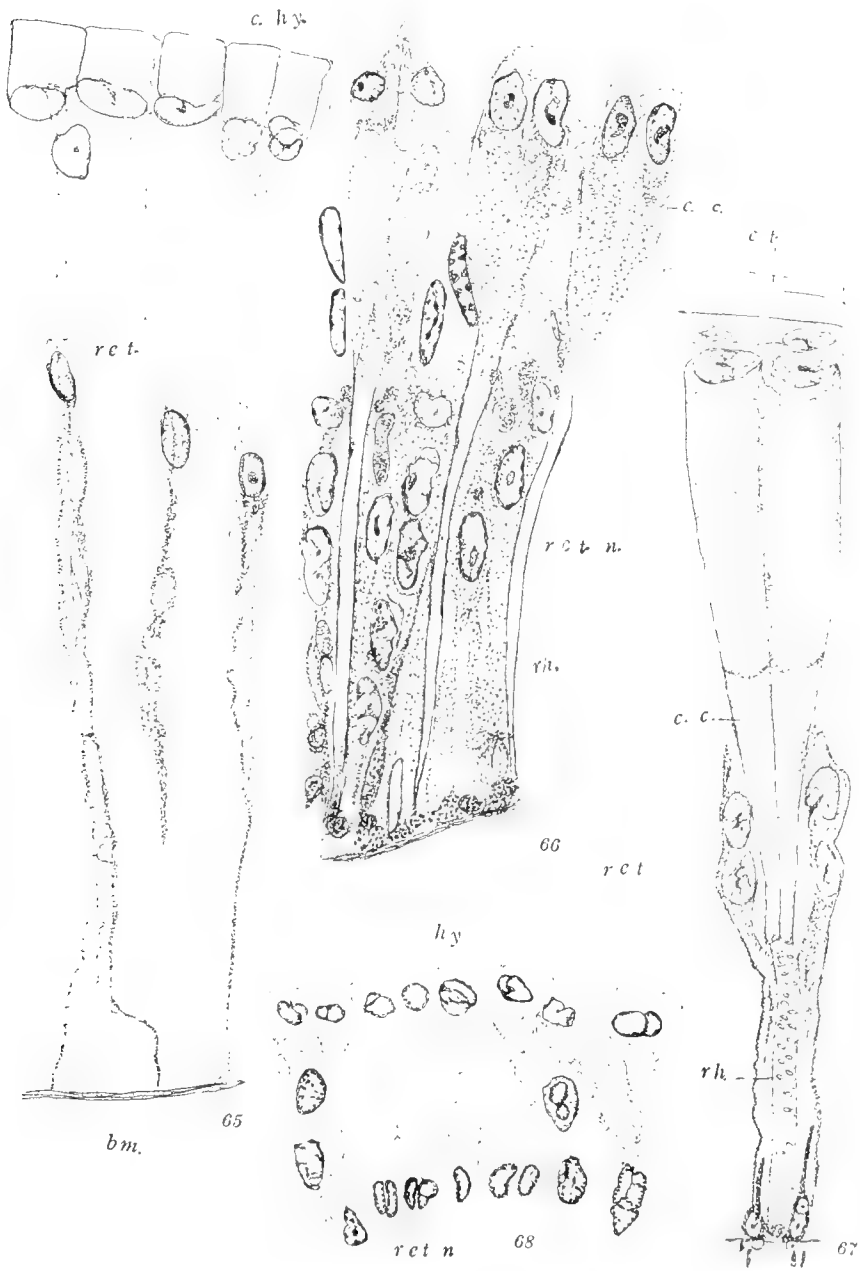


PLATE XIV

Fig. 69 Regenerating cones from eye shown in Fig. 20. Most of regenerated part of the eye occupied by abnormal tissue. Abnormal cells mingled with the normally regenerating structures. Compare Figs. 68-71. $\times 600$.

Fig. 70 Part of section of eye shown in Fig. 20. Most of the cells abnormal polymorphic nucleate cells except those comprising the hypodermis. $\times 450$.

Fig. 71 Right hand edge of Fig. 70 more highly magnified. Shows dividing hypodermal cells at upper edge and cells with polymorphic nuclei in interior. Three reticular nuclei at right edge of figure. $\times 1350$.

Fig. 72 Outline of section through stump shown in Fig. 43. Shows location of pigment spot with reference to other structures in stump. $\times 125$.

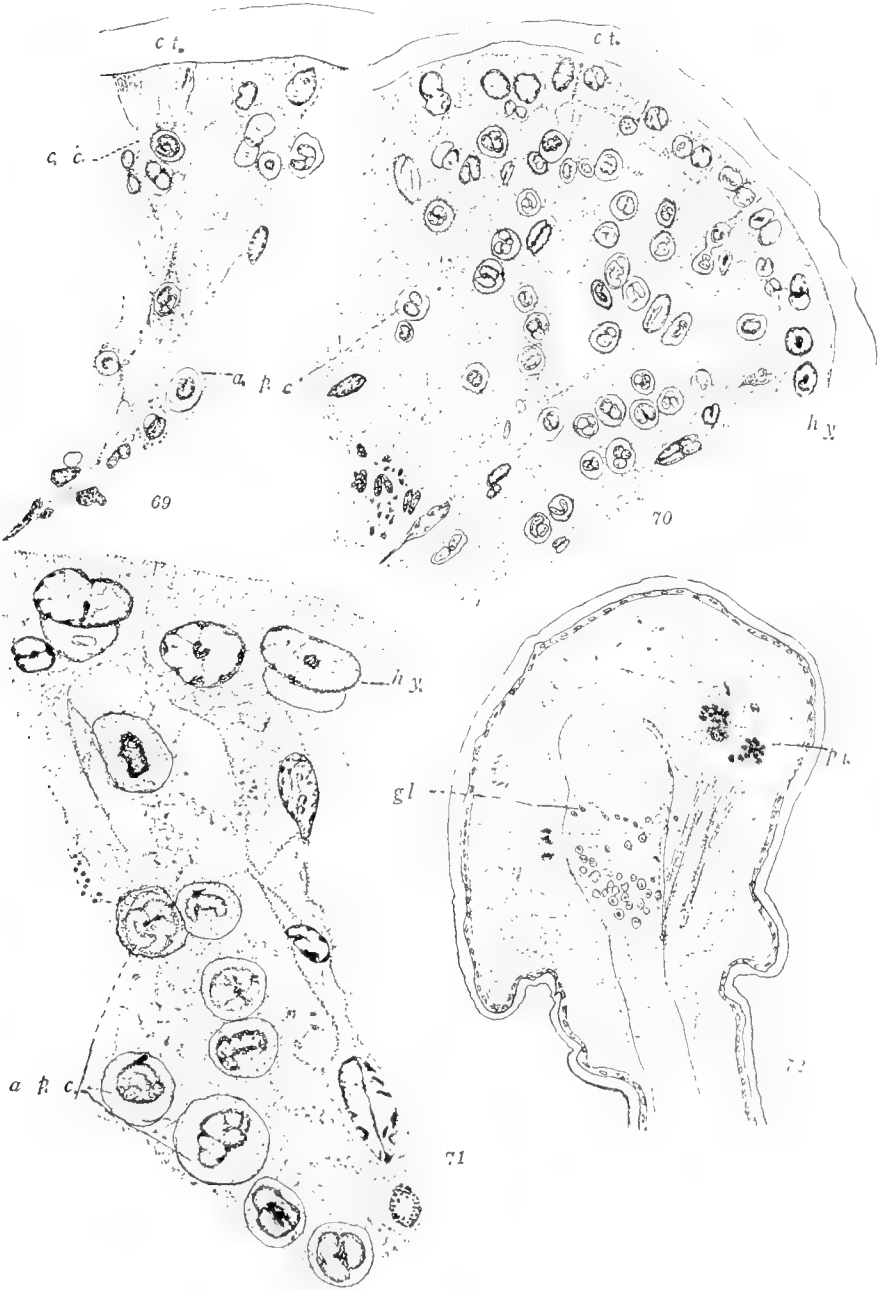


PLATE XV

Fig. 73 Detailed representation of pigment area shown in preceding figure. Shows collection of pigment cells within cysts. $\times 900$.

Fig. 74 Part of section from *Cambarus virilis* sixty-two and one-half hours after operation. In left part of figure lower part of cone and upper end of rhabdom. Remainder of figure occupied by chains of abnormal cells apparently developing from disintegrating retinulae. A few cells show pigment granules. $\times 910$.

Fig. 75 Group of cells which show polymorphic nuclei. *a*, Group of disintegrating retinulae from *Cambarus virilis* seventeen and one-half hours after operation; *b*, group of disintegrating retinulae from *Cambarus virilis* thirty-nine hours after operation; *c*, disintegrating retinulae from *Cambarus gracilis* sixteen days after operation; *d*, group of abnormal pigment cells from *Crangon* twenty-three days after operation; *e*, group of abnormal pigment cells from hermit crab sixty-seven days after operation; *f*, group of depigmented pigment cells from pigment cyst in eye of stump of *Palæmonetes* thirty days after operation; *g*, outline of crushed pigment body lying in same group with depigmented cells shown in *f*. $\times 900$.

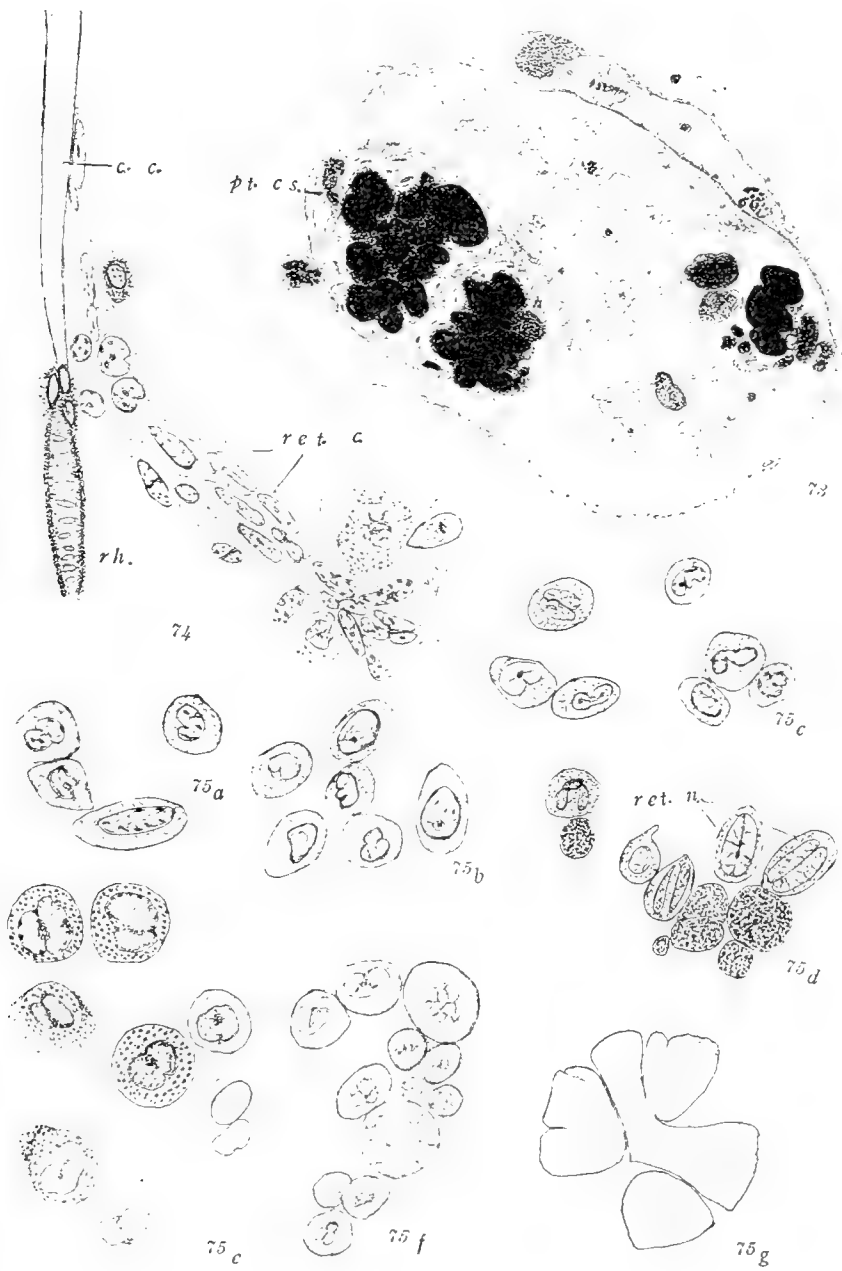


PLATE XVI

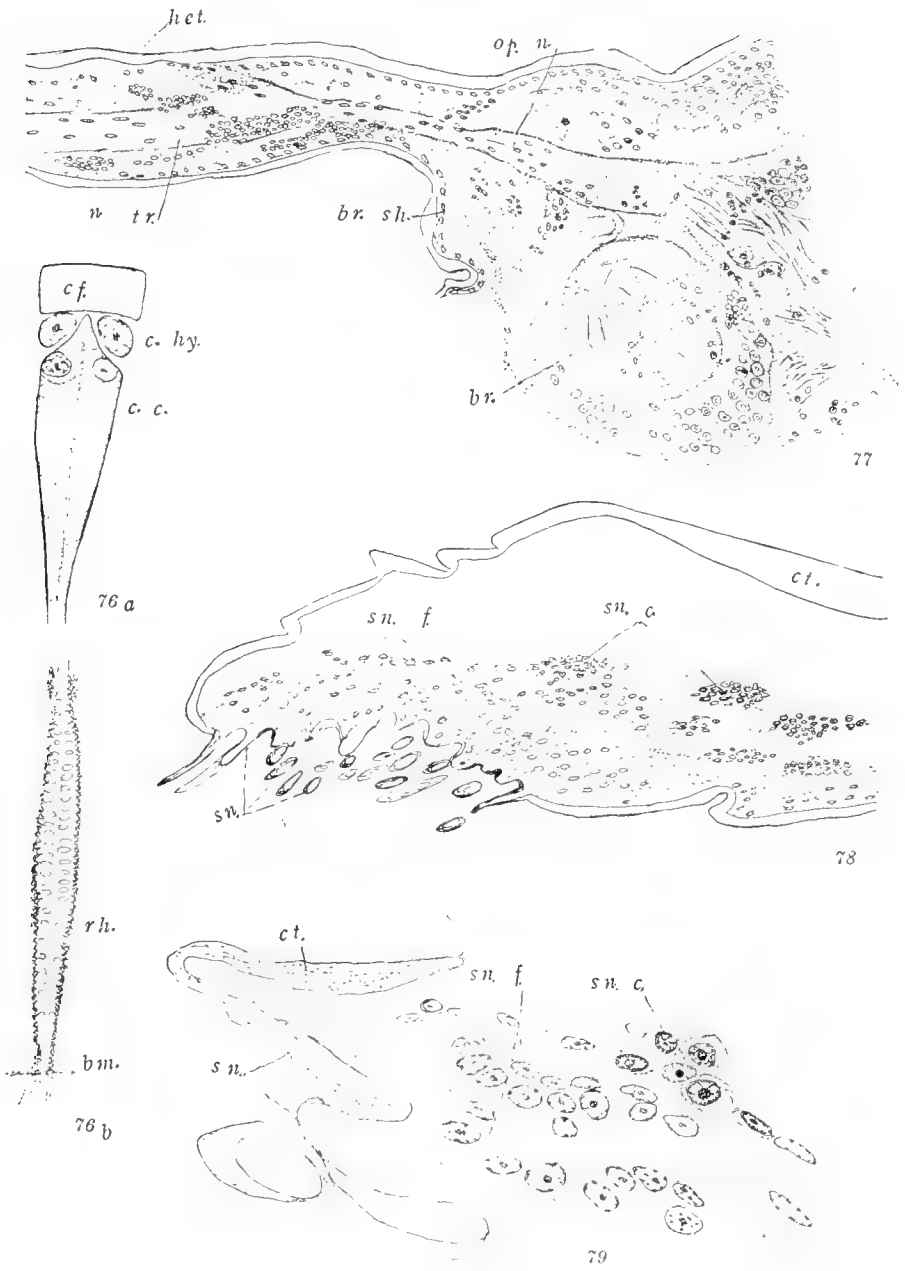
Fig. 76 *a*, Corneal facet and upper end of cone from fully regenerated ommatidium; *b*, fully regenerated rhabdom *a* and *b* both taken from regenerated hermit crab eye shown in Fig. 15. Ommatidia of hermit crabs much more slender than *Palaeomonetes* ommatidia. $\times 900$.

Fig. 77 Hermit crab, sixty-seven days. Section through brain and proximal end of heteromorphic appendage. Shows continuity of optic nerve and nerve trunk of appendage. Slightly diagrammatic. $\times 125$.

Fig. 78 Section through distal end of heteromorphic appendage, showing strands of fibers *sn.f.* extending to the sensory hairs, and groups of sensory cells, *sn.c.* $\times 125$.

Fig. 79 Detail drawing of small part of section shown in Fig. 78. Shows sensory cells and fibers *sn.f.* in connection with bases of two sensory hairs. $\times 750$.

MARY ISABELLE STEELE



ON SOME PHENOMENA OF COALESCENCE AND REGENERATION IN SPONGES¹

BY

H. V. WILSON

WITH FOUR FIGURES

I

In a recent communication I described some degenerative and regenerative phenomena in sponges and pointed out that a knowledge of these powers made it possible for us to grow sponges in a new way. The gist of the matter is that silicious sponges when kept in confinement under proper conditions degenerate in such a manner that while the bulk of the sponge dies, the cells in certain regions become aggregated to form lumps of undifferentiated tissue. Such lumps or plasmodial masses, which may be exceedingly abundant, are often of a rounded shape resembling gemmules, more especially the simpler gemmules of marine sponges (*Chalina*, *e. g.*), and were shown to possess in at least one form (*Stylotella*) full regenerative power. When isolated they grow and differentiate producing perfect sponges. I described moreover a simple method by which plasmodial masses of the same appearance could be directly produced (in *Microciona*). The sponge was kept in aquarium until the degenerative process had begun. It was then teased with needles so as to liberate cells and cell agglomerates. These were brought together with the result that they fused and formed masses similar in appearance to those produced in this species when the sponge remains quietly in aquarium. At the time I was forced to leave it an open question whether the masses of teased tissue were able to regenerate the sponge body.

During the past summer's work at the Beaufort Laboratory²

¹ Published with the permission of Hon. Geo. M. Bowers, U. S. Commissioner of Fisheries.

² I am indebted to the director of the station, Mr. H. D. Aller, for his kindly aid in supplying all facilities needed in the course of my investigation.

I again took up this question and am now in a position to state that the dissociated cells of silicious sponges after removal from the body will combine to form syncytial masses that have power to differentiate into new sponges. In *Microciona*, the form especially worked on, nothing is easier than to obtain by this method hundreds of young sponges with well developed canal system and flagellated chambers. How hardy sponges produced in this artificial way are and how perfectly they will differentiate the characteristic skeleton, are questions that must be left for more prolonged experimentation.

Taking up the matter where it had been left at the end of the preceding summer, I soon found that it was not necessary to allow the sponge to pass into a degenerative state, but that the fresh and normal sponge could be used from which to obtain the teased out cells. Again in order to get the cells in quantity and yet as free as possible from bits of the parent skeleton, I devised a substitute for the teasing method. The method adopted is rough but effective.

Let me briefly describe the facts for *Microciona*. This species (*M. prolifera* Verr.) in the younger state is incrusting. As it grows older it throws up lobes and this may go so far that the habitus becomes bushy. The skeletal framework consists of strong horny fibers with embedded spicules. Lobes of the sponge are cut into small pieces with scissors and then strained through fine bolting cloth such as is used for tow nets. A square piece of cloth is folded like a bag around the bits of sponge and is immersed in a saucer of filtered sea-water. While the bag is kept closed with the fingers of one hand it is squeezed between the arms of a small pair of forceps. The pressure and the elastic recoil of the skeleton break up the living tissue of the sponge into its constituent cells, and these pass out through the pores of the bolting cloth into the surrounding water. The cells, which pass out in such quantity as to present the appearance of red clouds, quickly settle down over the bottom of the saucer like a fine sediment. Enough tissue is squeezed out to cover the bottom well. The cells display amœboid activities and attach to the substratum. Moreover they begin at once to fuse with one

another. After allowing time for the cells to settle and attach, the water is poured off and fresh sea-water added. The tissue is freed by currents of the pipette from the bottom and is collected in the center of the saucer. Fusion between the individual cells has by this time gone on to such an extent that the tissue now exists in the shape of minute balls or cell conglomerates of a more or less rounded shape looking to the eye much like small invertebrate eggs. Microscopic examination shows that between these little masses free cells also exist, but the masses are constantly incorporating such cells. The tissue in this shape is easily handled. It may be sucked up to fill a pipette and then strewn over cover glasses, slides, bolting cloth, watch glasses, etc. The cell conglomerates which are true syncytial masses throw out pseudopodia all over the surface and neighboring conglomerates fuse together to form larger masses, some rounded, some irregular. The details of later behavior vary, being largely dependent on the amount of tissue which is deposited in a spot, and on the strength of attachment between the mass of tissue and the substratum.

Decidedly the best results are obtained when the tissue has been strewn rather sparsely on slides and covers. The syncytial masses at first compact and more or less rounded, flatten out becoming incrusting. They continue to fuse with one another and thus the whole cover glass may come to be occupied by a single incrustation, or there may be in the end several such. If the cover glass is examined at intervals, it will be found that differentiation is gradually taking place. The dense homogeneous syncytial mass first develops at the surface a thin membrane with underlying connective tissue (collenchyma). Flagellated chambers make their appearance in great abundance. Canals appear as isolated spaces which come to connect with one another. Short oscular tubes with terminal oscula develop as vertical projections from the flat incrustation. If the incrustation be of any size it produces several such tubes. The currents from the oscula are easily observed, and if the cover glass be mounted in an inverted position on a slide the movements of the flagella of the collar cells may be watched with a high power (Zeiss 2 mm.).

This degree of differentiation is attained in the course of six or seven days when the preparations are kept in laboratory aquaria (dishes in which the water is changed answer about as well as running aquaria). Differentiation goes on more rapidly when the preparation is hung in the open harbor in a live-box (a slide preparation inclosed in a coarse wire cage is convenient). Sponges reared in this way have been kept for a couple of weeks. The currents of water passing through them are certainly active and the sponges appear to be healthy. In such a sponge spicules are present, but some of these have unquestionably been carried over from the parent body along with the squeezed out cells.

The old question of individuality may receive a word here. *Microciona* is one of that large class of monaxonid sponges which lack definite shape and in which the number of oscula is correlated simply with the size of the mass. While we may look on such a mass from the phylogenetic standpoint as a corm, we speak of it as an individual. Yet it is an individual of which with the stroke of a knife we can make two. Or conversely it is an individual which may be made to fuse with another, the two forming one. To such a mass the ordinary idea of the individual is not applicable. It is only a mass large or small having the characteristic organs and tissues of the species but in which the shape of the whole and the number of the organs are indefinite. As with the adult so with the lumps of regenerative tissue. They have no definiteness of shape or size, and their structure is only definite in so far as the histological character of the syncytial mass is fixed for the species. A tiny lump may metamorphose into a sponge, or may first fuse with many such lumps, the aggregate also producing but a single sponge although a larger one. In a word we are not dealing with embryonic bodies of complicated organization but with a reproductive or regenerative tissue which we may start on its upward path of differentiation in almost any desired quantity. A striking illustration of this nature of the material is afforded by the following experiment. The tissue in the shape of tiny lumps was poured out in such wise that it formed continuous sheets about one millimeter thick. Such sheets were then cut into pieces, each about one cubic millimeter. These

were hung in bolting cloth bags in an outside live-box. Some of the pieces in spite of such rough handling metamorphosed into functional sponges.

Even where the embryonic bodies of sponges have a fixed structure and size, as in the case of the ciliated larva, the potential nature as displayed in later development, is not fixed in the matter of individuality. Such a body (see p. 10) may form a single individual or may fuse with some of its fellows to form a larger individual differing from the one-larva sponge only in size. It is then in spite of its definiteness of shape and size, essentially like a lump of regenerative tissue in that whether it develops into a whole sponge or a part of a sponge depends not on its own structure but on whether it is given a good opportunity of fusing with a similar mass. A parallel case to the coalescence of larvæ is afforded by the gemmules of fresh water sponges. Mr. M. E. Henriksen in a manuscript account submitted to me a year ago, describes the fusion of gemmules to form a single sponge.

In the preceding description I have passed over the question as to the precise nature of the cells which combine to form the masses of regenerative tissue. On this point as on the histological details in general I hope to have more to say later. Nevertheless the phenomena are so simple that observation of the living tissue reveals much, probably indeed all that is of fundamental importance. If a fairly dense drop of the squeezed out tissue be mounted at once and examined with a high power (Zeiss 2 mm., comp. oc. 6), the preparation is seen to consist of fluid (sea-water) with a few spicules and myriads of separate cells. The cells fall into three classes.

1 The most conspicuous and abundant are spheroidal, reddish, densely granular, and about (8μ) in diameter. These cells which can be nothing but the unspecialized, amœboid cells of the mesenchyme (amœbocytes or archæocytes), put out hyaline pseudopodia that are sometimes elongated, more often rounded and blunt.

2 There is also a great abundance of partially transformed collar cells, each consisting of an elongated body with slender flagellum. The cell is without a collar, the latter doubtless hav-

ing been retracted. In the freshly prepared tissue the flagella are vibratile, the cells moving about. Soon however the flagellum ceases to vibrate.

3 The third class is not homogeneous. In it I include more or less spheroidal cells ranging from the size of the granular cells down to much smaller ones. Many of these are completely hyaline, while others consist of hyaline protoplasm containing one or a few granules.

Fusion of the granular cells begins immediately and in a few minutes time most of them have united to form small conglomerate masses which at the surface display both blunt and elongated pseudopodia. These masses soon begin to incorporate the neighboring collar and hyaline cells. One sees collar cells sticking fast by the end of the long flagellum to the conglomerate mass. Other collar cells are attached to the mass by short flagella. Still again only the body of the collar cell projects from the mass while there is no sign of the flagellum. Similarly spheroidal hyaline cells of many sizes are found in various stages of fusion with the granular conglomerate. In such a preparation the space under the cover glass is soon occupied by innumerable masses or balls of the kind just described, between which continue to lie abundant free cells, some collar cells, others hyaline. Practically all the granular cells go to make up the balls. The play of pseudopodia at the periphery of such balls, which results in the incorporation of free cells and in the fusion of balls to form larger masses, is easily watched. Along with such a cover glass preparation it is convenient to have some of the squeezed-out tissue in a watch glass of sea-water. In the watch glass preparation it is instructive to watch with a two-thirds or one-half objective the fusion of the cell conglomerates to form masses like those strewn on covers, slides, etc. (p. 3).

These observations on the early steps in the formation of the masses of regenerative tissue make it plain that such masses are composed chiefly of the spheroidal, granular cells (amœbocytes or archæocytes), but that nevertheless other cells, collar cells and more or less hyaline cells also enter into their composition. I may recall the fact that in the formation of regenerative masses in a

degenerating sponge,³ the evidence from sections, which is the only evidence available in the case, points to the conclusion that the collar cells help to form the syncytial tissue of the masses. The question of interest lying at the heart of this matter may be so formulated: can particles of the Microciona protoplasm differentiate into functional collar cells and, when the occasion arises, change back into unspecialized masses capable of combining with other masses of unspecialized protoplasm to form a regenerative body? The facts to which I have just alluded support this idea, and indicate that the immediate problem is one worth pursuing farther as a good case of temporary differentiation of protoplasm in the metazoa analogous to the temporary specialization of the cell individual which occurs in such colonial protozoa as *Protospongia*.⁴

As far as the amœbocytes are concerned it is certain that they have great regenerative power. Weltner in a recent paper⁵ has emphasized the importance of these unspecialized cells in the processes of growth and regeneration. His conclusions which refer directly to fresh water sponges, are that in a growing sponge, in a sponge regenerating new organs after its winter period of simplification, and in the regeneration of a sponge from a cutting, the amœbocytes are the all-powerful elements in that they give rise to all the new tissues formed. He further alludes to the fact that such reproductive bodies as the gemmules of fresh water sponges and the buds of *Tethya* (according to Maas) are only groups of amœbocytes; further that the gemmules of *Tedania* and *Esperella* described by Wilson as developing into ciliated larvæ, and the similar bodies found by Ijima in hexactinellids, are such groups. I may add that the presence of such groups of unspecialized cells in the hexactinellids has recently been confirmed by the master in sponge-morphology, F. E. Schulze, who recognizes the probability of their reproductive nature and gives

³ A new method by which sponges may be artificially reared, *Science*, n. s., vol. xxv, no. 649, 1907

⁴ Metschnikoff, *Embryologische Studien an Medusen*, p. 147, 1886.

⁵ Spongilliden-studien V. Zur Biologie von *Ephydatia fluviatilis* und die Bedeutung der Amœbocyten für die Spongilliden. *Archiv für Naturgeschichte*, 73 Jahrg., 1 Bd., 2 Heft, 1907.

them a new name, that of *sorites*.⁶ It is clear then that in many sponges reproductive bodies are formed by the association of unspecialized amœboid cells. But there is nothing in this fact which precludes the possibility that the groups of amœbocytes are in part recruited from transformed collar cells and other tissue cells, such as pinacocytes (flat cells of canal walls), that have undergone regressive differentiation into an unspecialized amœboid condition.

Cells analogous to the amœbocytes of sponges are found elsewhere in the metazoa, *e. g.*, in the ascidians.⁷ It would be interesting to know what capacity, if any, for development they have, when freed from the parent (bud) and collected together in seawater.

II

I shall here briefly record some experiments which gave only negative results but which under circumstances admitting of a wider choice of species, ought to yield returns of value. These experiments were based on the assumption that if the dissociated cells of a species will recombine to form a regenerative mass and eventually a new sponge, the dissociated cells of two different species may be made to combine and thus form a composite mass bearing potentially the two sets of species-characteristics. It is clear that such an organism would be analogous to one produced by an association of the blastomeres of the two species. Pending the successful carrying out of this experiment, it would be idle to discuss further the nature of the hypothetical dual organism.

In my own experiments three sponges were used: *Microciona*, *Lissodendoryx* and *Stylotella*. The three are all monactinellids, but *Microciona* is the only one in which the skeleton includes any considerable amount of horny substance. Dissociated cells of *Microciona* and *Lissodendoryx* were mixed, and again dissociated cells of *Microciona* were mixed with those of *Stylotella*. In each case the experiment was performed at two different times, and a considerable number of admixtures, in watch glasses and on

⁶ *Wissensch. Ergebn. d. Deutsch. Tiefsee-Exp.* 1898-99. Hexactinellida, pp. 213-15. Jena, 1904.

⁷ *Comp. Hjort's and Lefevre's papers on budding in ascidians.*

cover glasses, was made. The preparations were examined at short intervals with the microscope. The cells of these three species are colored very differently, and are therefore easily distinguished, at least as soon as fusion sets in and little masses of cells begin to be formed. In all the experiments the cells and cell-masses of a species combined, and not the cells of different species. Thus in the admixture of *Microciona* and *Lissodendoryx*, *Microciona* regenerative masses and *Lissodendoryx* regenerative masses were produced. Similarly when *Microciona* and *Stylotella* cells were mixed, the resultant masses were pure, some *Microciona*, some *Stylotella*. The *Microciona* masses in these experiments were hardy. They continued to develop and in some preparations metamorphosed. The cell masses of the other two species while they reached a considerable size were not hardy, most dying soon although some began the process of metamorphosis.

These three species are so unlike that there was little ground in the beginning for the expectation that coalescence would take place. Possibly as in the cases where fusion of egg and sperm of different species is induced through some alteration in the physiological state of the protoplasm, so the regenerative cells and cell masses of different species may be made to combine under abnormal conditions. The more promising task is however to find allied species and subspecies, the regenerative tissue of which will combine under natural conditions. Such forms, I take it, should be sought among the horny sponges and the monactinellids with abundant horny matter.

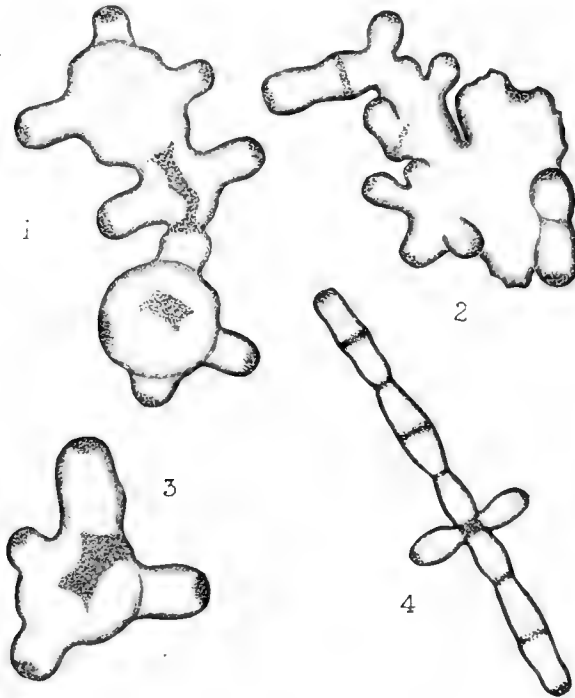
III

The tendency to fuse so vigorously displayed by the cells and cell masses of regenerative tissue led me to examine into the power that larvæ have to fuse with one another and the capacity for development in the resultant mass. Delage and others have remarked on the not infrequent occurrence of fusion between sponge larvæ. Delage³ says that he has often observed two or

³ Embryogénie des Éponges. Arch. de Zool. Exp. et Gén., p. 400, 1892.

several larvæ unite to form a single sponge "which has from the start several cloacas."

I find that this power to fuse displayed by the larvæ is one that is easy to control. Fusion between larvæ will readily take place if they are brought in contact at the critical time when the ciliated



Figs. 1, 2, 3, 4 Composite masses produced by the fusion of larvæ. The stippled ends and areas are in nature blue, and represent the ends of the component larvæ. The body of the mass is white. Fig. 1 shows a mass composed of four larvæ which has just united with a mass composed of five or six larvæ. In Fig. 2 more than ten, probably about twenty, larvæ have combined. In Fig. 3 about six larvæ have combined. In Fig. 4 the original quadruple mass composed of four radiately arranged larvæ, has been extended in one direction by the addition of a pair of larvæ, and in the opposite direction by the addition of two pairs of larvæ. Figs. 1 and 3 $\times 44$; Figs. 2 and 4 $\times 22$.

epithelium is being replaced by the permanent flat epithelium. At this time they will fuse in twos or threes or in larger number up to and over one hundred (Figs. 1-4). The smaller composite masses composed of as many as five or six larvæ metamorphose into perfect sponges. The larger masses composed of many

larvæ did not metamorphose in my experiments but experience with the regenerative tissue suggests that such masses would metamorphose if certain mechanical difficulties due to the great size of the mass were removed. Possibly this might be accomplished by cutting a flattened sheet composed of some hundred larvæ (such as I have produced) into pieces and inducing the pieces to metamorphose separately.

I may now describe some of the details in this process of larva-fusion. In a species of *Lissodendoryx* used the larva is of the following character. It has the usual ovoidal shape with a posterior protuberant non-ciliated pole. The anterior pole is somewhat truncated and is sparsely ciliated. The rest of the body bears the usual thick covering of cilia. As seen with reflected light the bulk of the body is dead white, the posterior pole deep blue, and the anterior pole bluish. This coloration is not absolutely fixed for the species, but the larvæ used in my coalescence experiments were all of this character. Within twenty-four hours after liberation the ciliated larvæ are creeping (remaining in contact with the bottom as they swim) over the bottom of the dish. Some are now put in deep round watch glasses and with pipette and needle coaxed together into a clump. Fusion soon begins and on the next day plenty of composite larvæ are present. The larvæ fuse endwise, for the most part in pairs. The compound larva so produced owing to its weight has a very feeble locomotory power. Using pairs that are nearly motionless, larvæ may be brought together (coaxed with needle) and arranged in a desired position on a cover glass for instance. In successful cases fusion results before the separate masses move apart. In this way, selecting an instance, I have added to one arm of a quadruple mass a pair of larvæ, and to the opposite arm two pairs (Fig. 4).

For the purpose of bringing about the fusion of many larvæ the following simple method is convenient. Suppose that we have the larvæ in a paraffine-coated dish, and they are in a late "creeping" stage. Small excavations, 2-3 mm. deep and 4-5 mm. wide, are now made in the paraffine, and with the pipette the larvæ are driven into the holes. They lie here in numbers up to and over one hundred, crowded together and heaped upon one another.

Fusion begins soon and the larvæ are gradually converted into a flattened cake. The larger cakes thus made measured four by three millimeters. The body of such a cake is a continuous flattened mass in which there is no indication of the component larvæ, but the rounded ends of the larvæ that have last fused with the general mass remain for a time distinguishable. Owing to their blue coloration the ends of the larvæ may be recognized in these and the other compound masses even after the outline of the larva has been completely lost.

As already stated the smaller compound masses metamorphose without difficulty. The coalesced larvæ may be made to attach to cover glasses, slides, etc. Larger masses composed of about twenty larvæ underwent a partial metamorphosis. Such masses were laid upon bolting cloth to which they readily attached. The largest masses were hung in small bolting cloth bags in a live box. Whether owing to bad handling or more probably to some inherent difficulty, they did not metamorphose but soon died.

The ease with which larvæ of the same species may be made to fuse together suggests that larvæ of different species might likewise be induced to coalesce. Some experiments along this line could not fail to be of interest.

IV

In the tendency to fuse with the production of a plasmodium, the dissociated cells of sponges resemble the amœbocytes (amœbulæ) of the mycetozoa and *Protomyxa*. The regenerative power of the plasmodium has an interest both theoretical and economic in itself. But it is the tendency to fuse displayed by the cells that have been forcibly broken apart, which constitutes the fact of most general physiological importance. Discarding for the moment the word "cell" and speaking of the protoplasm of a species as a specific substance, the phenomena may be restated to advantage in the following way.

A mass of sponge protoplasm in the unspecialized state typically exhibits pseudopodial activities at the surface. In lieu of more precise knowledge it is useful to regard the pseudopodia as structures which explore and learn about the environment. On coming

EQUILIBRIUM OF ANIMAL FORM¹

BY

HANS PRZIBRAM

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WITH TEN FIGURES

If the equilibrium of a mass be disturbed, the body will alter its position in regard to the surrounding neighborhood till it again gets into a position of equilibrium. If the form of an animal be altered through amputation of certain parts, the equilibrium of mass may or may not be altered therewith, the animal either being able to maintain its position or having to alter its posture for readjusting its equilibrium. But this is not all that may happen: it may restore its *form*, too, after some time, thus tending toward a new equilibrium of *form*, till it has reached a new stable condition.

This may involve three regulatory processes: Regeneration of lost parts from the cut surface; reduction of existing parts in contact with the cut surface; compensation at parts of the body not touched by the amputation.

The study of regeneration has long received much attention; of late reduction too has been studied more fully, especially in the lower animals, whilst compensation as a means of restoring animal form seems first to have been pointed out by me. Its study has been taken up especially in America, where Zeleny found additional cases (observed independently of my work), and Wilson, Morgan and Emmel have studied different aspects of compensatory regulation in crustaceans.

Having found the principle of compensatory regulation illustrated in the chelæ of *Alpheus*, I have been looking for other analogous cases and have found the same process in other crustaceans, especially *Callinassa* and the common Crabs, *Portunus* and

¹ Read before the International Congress of Zoology at Boston on August 22, 1907.

Carcinus, as I demonstrated at the last session of the German Naturalists' Association. It will be remembered that this compensatory regulation consists in the hypertypical growth of the smaller claw of the first pair of thoracic limbs, after autotomic removal of the big chela, whilst a hypotypical small chela regenerates. That such a "transposition" or "reversal" need not be restricted to this pair of appendages I am now able to show in *Typton spongicola* (Fig. 1), where the *second* pair of thoracic limbs is developed into asymmetrical chelæ. After removal of the bigger chela, which, by the way, may be situated normally at the right or at the left side of the body, reversal of the chelæ of this second pair is brought about (Fig. 2), the process being in all respects analogous to that in the first pair of chelæ in *Alpheus*.

But not only may the means of regeneration and compensation be clearly shown to occur in this case, but also reduction is involved to an appreciable degree, especially if the crayfish is starved during the experiment. Then each moult shows the shedding of a smaller skin and the animal is at the end of the transposition in all dimensions smaller than at the time of the operation. Thus, as in the lowest animals, a proportionate diminution of the whole form may be produced as regulation proceeds, the only difference with Morgan's "morphallaxis" lying in the bigger fragment necessary for reconstruction.

A curious instance of "compensatory reduction" was met in some experiments on cutting the nerves in crabs. As Morgan has reported, the chela generally degenerates after this operation. In a few cases, however, I was fortunate enough to get a further growth of the limb. In these the terminal joint of the big or crushing claw was removed (Fig. 3), and in one instance regenerated in a rather reduced state; but also the corresponding dactylopodite of the smaller or nipping claw lost its differentiation (Fig. 4).

Compensatory reduction may also occur in animals other than crustaceans and in other regions than in the chelæ. Megusar, working on regeneration in beetles (Coleoptera), amputated one of the two slightly differing jaws of the *Hydrophilus* larva. Whilst there are normally two teeth on the inner side of each jaw (Fig. 5) the larva appeared after the moult succeeding the amputation

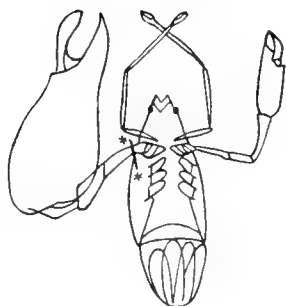


FIG. 1.

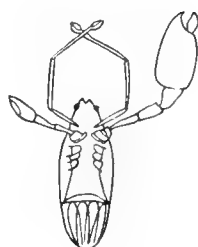


FIG. 2.

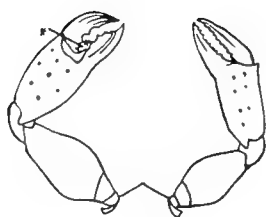


FIG. 3.

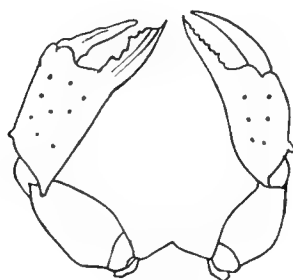


FIG. 4.

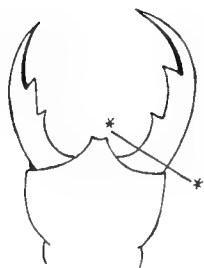


FIG. 5.

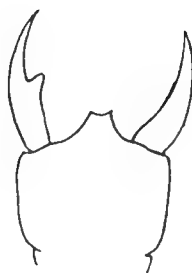


FIG. 6.

of but one jaw with but *one* tooth on the non-operated jaw, the regenerating one showing no teeth (Fig. 6).

A tendency toward a quick restoration of a symmetrical condition was also found several times in experiments of Miss Zuelzer on the regeneration and moulting periods of the isopod, *Asellus aquaticus*. When both long antennæ were removed, simultaneously, but at different levels (Fig. 7) they would be apt to appear regenerated to an *equal* length (Fig. 8) even after the next moult, though not having yet attained their normal length. In some way these conditions may be related to the different rate with which appendages regenerate from different levels.

It is not necessary that the two correlated parts be symmetrically situated. The male of the water-newt, *Triton vulgaris*, produces in its state of courtship a crest along its back and around its tail, which has a ragged appearance (Fig. 9). Kammerer cut the tail off to test the regenerative power of the secondary sexual characters. He found that at first the tail appears with smooth not ragged edges. But this is not all: the crest on the back had also lost its ragged appearance, thus conforming with the outline of the new dorsal rim of the tail (Fig. 10). It is not shed or resorbed, but keeps the height of the courtship crest.

The object of my paper is to emphasize the similarity of these processes of regeneration, reduction and compensation, in lower and higher animal forms, and their relation to the reestablishment of the equilibrium of animal form.



FIG. 7.



FIG. 8.

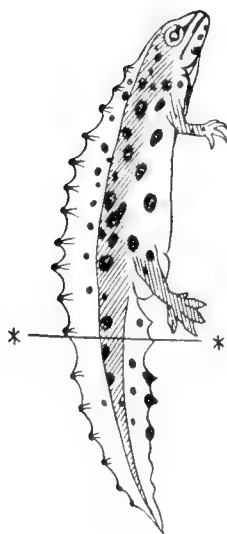


FIG. 9.



FIG. 10.

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THE EFFECT OF DEGREE OF INJURY, SUCCESSIVE INJURY AND FUNCTIONAL ACTIVITY UPON REGENERATION IN THE SCYPHOMEDUSAN, *CASSIOPEA XAMACHANA*¹

BY

CHARLES ZELENY

WITH FOUR FIGURES

INTRODUCTION

The present study is a part of a series of experiments whose object is the investigation of some of the internal factors controlling regeneration in several representative forms. The factors taken up in *Cassiopea* are the degree of injury, successive removal of a part and rhythmical pulsation of the disk. It is found that removal of six of the eight oral arms constitutes the most favorable degree of injury for the regeneration of each arm, and that from this optimum there is a decrease in both directions. The data for successive injury show a greater rate of regeneration of the margin of the disk after the second removal, than after the first. A comparison of the rate of regeneration of the margin in cases where the disk was made to pulsate rhythmically with cases without pulsation shows no advantage in favor of the pulsating ones, but rather a retardation.

¹ Contributions from the Zoölogical Laboratory of Indiana University. No. 92.

I am indebted to the Carnegie Institution of Washington, for the privilege of working at their laboratory at Tortugas. To Dr. A. G. Mayer, the director, I am under obligation for many kindnesses and especially for suggestions in connection with the work.

The present paper is the fourth of a series dealing with the internal factors controlling the rate of regeneration. The other three papers are: *a* A study of the rate of regeneration of the arms in the brittle-star, *Ophioglypha lacertosa*. *Biological Bulletin*, vol. vi, no. 1, December, 1903; *b* The relation of the degree of injury to the rate of regeneration. *Journal of Experimental Zoölogy*, vol. ii, no. 3, August, 1905; *c* Some internal factors connected with the regeneration of the chelæ in the Gulf-weed Crab, *Portunus Sayi*. In press. Carnegie Institution.

METHOD

An abundance of material of all sizes was obtained in the moat of the fort at Tortugas. The animals were found to live very well in glass dishes in the laboratory with but a single change of water each day. Every effort was made to keep all conditions, except the ones used in comparison, as much alike as possible. None of the animals were fed during the experiments and as a result both normal and mutilated specimens decreased in size. Notwithstanding this decrease the animals remained healthy and regenerated readily even after severe injuries. The starving of the animals was necessary because it is impossible to feed equal amounts to animals with different degrees of mutilation.

DATA AND RESULTS

1 The Influence of Degree of Injury on the Rate of Regeneration

The rate of regeneration of a single oral arm in each of the following cases was determined:

- a* one arm removed at its base;
- b* two arms removed at their bases;
- c* four arms removed at their bases;
- d* six arms removed at their bases;
- e* all eight arms removed at their bases;
- f* whole mouth apparatus removed.

Case *f* is not an integral part of the series because the arms do not regenerate from the same level as in cases *a* to *e*.

Five groups each similar to the above were obtained, the members of a group being approximately equal in size.

The data as given in Table I show that the rate of regeneration of an oral arm is the lowest when that arm alone is removed. From this minimum the rate increases up to the optimum at six removed arms. It then decreases being less when eight arms are removed. The first five cases in the table are strictly comparable because in each the arms were removed at the base, and therefore, the regeneration is from the same surface in each. In the sixth or last case *f* this is not true. Here the whole mouth apparatus, including the arms, was removed and regeneration is not from

the same surface as before. The measurements were made after death. The animals were stupefied in CO₂ sea-water and preserved in 10 per cent formalin.

TABLE I

	ONE ARM			TWO ARMS			FOUR ARMS			SIX ARMS			EIGHT ARMS			WHOLE MOUTH APPARATUS*		
	Disk diam.	Lg. of reg. arm	Sp. amt.	Disk diam.	Av. lg. reg. arm	Sp. amt.	Disk diam.	Av. lg. reg. arms	Sp. amt.	Disk diam.	Av. lg. reg. arms	Sp. amt.	Disk diam.	Av. lg. reg. arms.	Sp. amt.	Disk diam.	Av. lg. reg. arms	Sp. amt.
Group A	10.5	.5	.048	8.4	1.5	.179	11.2	2.0	.179	9.0	2.5	.278	10.8	2.0	.185	10.8	1.6	.148
Group B	17.0	1.0	.059	21.0	2.5	.119	15.5	3.0	.194	16.5	2.5	.151	17.5	2.5	.143	16.5	2.0	.121
Group C	22.5	1.8	.080	30.5	2.5	.082	24.0	2.5	.104	28.5	3.5	.123	25.0	2.5	.100	25.0	1.5	.060
Group D	29.0	1.0	.034	29.0	1.75	.060	35.0	4.7	.134	26.0	2.85	.110	28.0	2.0	.072	28.0	2.15	.077
Group E	42.0	3.0	.071	42.0	5.0	.119	36.0	4.0	.111	37.0	4.5	.122	34.5	3.0	.087	32.5	5.0	.154
Average		1.66	.058		2.65	.112		3.24	.144		3.17	.157		2.40	.117		2.45	.112

* Not strictly comparable with the others because the regenerating surface is at a deeper level.

The average lengths of the regenerated arms are 1.66 mm. for the individuals with one removed arm, 2.65 for those with two removed arms, 3.24 for those with four, 3.17 for those with six, 2.40 for those with eight and 2.45 for those with the whole mouth apparatus removed. Since the individuals of a group are not exactly alike, and since the amount of regeneration is dependent on the size, the specific amount, *i. e.*, the regenerated length divided by the disk diameter was obtained in each case. The average specific amount of regeneration for each degree of injury is given in Table I in *italic type*. It is seen that from a minimum for the case with the lowest injury it increases to an optimum when six arms are removed, beyond which it again decreases. Thus with one removed arm the specific amount is .058, with two arms removed it is .112, with four .144, with six .157, and with eight .117.

A series of individuals with different extents of removed mar-

gins was studied with respect to the question of relation of degree of injury to the rate of regeneration, but because of distortions involving the whole umbrellar region, no adequate data were obtained.

2 *The Effect of Successive Injury on the Rate of Regeneration*

The study of successive injury was confined to the margins. As in the last mentioned case there was considerable trouble with distortion of the disk. Eight individuals however were without distortion and could be used for the present purposes. The experiments on successive injury come under two heads. In one series a number of individuals of a size were chosen. In half of these the whole margin was removed and allowed to regenerate for twenty-nine days, at the end of which time it had nearly completed its regeneration. This margin was then removed for the second time at the same hour that it was removed for the first time in the others. After twelve days the animals were killed and a measurement of the new margins gave a direct comparison of first and second regeneration as shown in Table II.

TABLE II

<i>Width of regenerated margin in millimeters</i>	
First regeneration	Second regeneration
.5	.8
.2	1.6
.2	
—	—
Average..... .3	1.2

The second regeneration shows a decided advantage over the first.

In a second series the first and second regenerations were compared within single individuals. A part of the margin of each individual was removed, and after it had nearly completed its regeneration it was removed again at the same time that a similar segment from another part of the circumference was removed for the first time. A direct elimination of individuality was thus obtained.

On account of individual differences in the method each case is described separately. In each, however, the first operation

came on June 17, and the second on July 16, twenty-nine days later. Twelve days were allowed after the second operation.

In individual A about one-sixth of the margin was removed. The regenerated margin was 2.0 mm. in width twenty-nine days later. It was then removed a second time and a similar segment was removed from a part of the circumference which had not been injured. Twelve days later the animal was killed and showed a second regeneration width equal to 1.8 mm. and a first regeneration width of 1.6 mm.

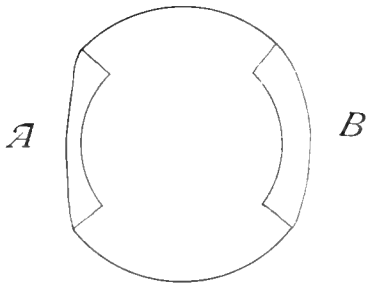


FIG. 1

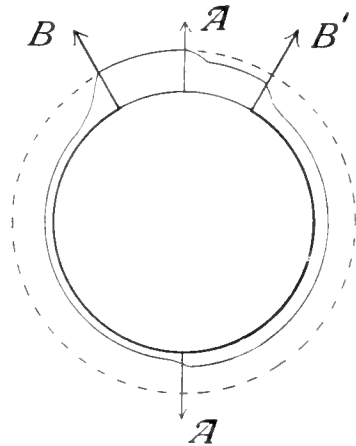


FIG. 2

Fig. 1 Outline of disk of *Cassiopea xamachana* in which segment B is a second regeneration and segment A a first regeneration of the margin.

Fig. 2 Outline of the disk of *Cassiopea xamachana* in which the unbroken line between B' and lower A is a second regeneration of the margin to be compared with a first regeneration shown between B and lower A.

In individual B the character of the operation was the same as in A but the removed portion of the margin was one-fourth of the whole for each regeneration, and the regenerated margin was 2.7 mm. wide as compared with the uninjured 4.2 mm. In this case the second regeneration width is 2.0 mm., and the first 1.1 mm. The relative widths are shown in Fig. 1, where A represents the first regeneration and B the second.

In individual C as shown in Fig. 2 in semidiagrammatic form the original outer circumference is shown by the dotted line and

outer unbroken line between B and A. At the first operation the half of the margin shown in the figure by the part to the right of a line between A and A' was removed. This was allowed to regenerate until it had a width of 3.5 mm. as compared with 4.5 mm. in the uninjured margin. The second operation was then made and consisted of a cut along the heavy line between B and B' including five-sixths of the whole margin. This second operation included a strip of regenerated margin, and an equal strip of margin which had previously been uninjured. After twelve days the animal

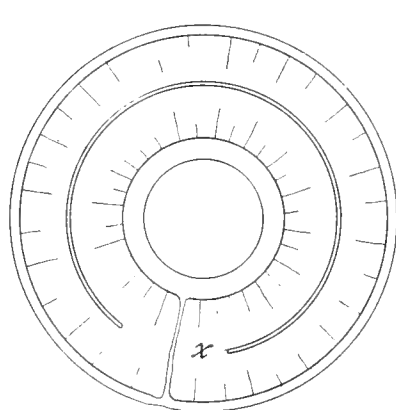


FIG. 3

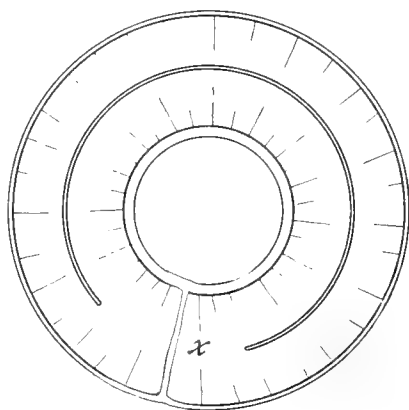


FIG. 4

Fig. 3 Regeneration of inner and outer margins in a *non-pulsating* individual of *Cassiopea xamachana*. Compare with Fig. 4.

Fig. 4 Regeneration of inner and outer margins in a *pulsating* individual of *Cassiopea xamachana*. Compare with Fig. 3.

was killed and the average width of the second regeneration (B' to lower A) was 1.4 mm. as compared with a first regeneration width (B to lower A) of .8 mm.

In all three individuals there is a well marked difference between the second and the first regeneration in favor of the former. The character of the operation to a very large extent eliminates chance of individual error, and makes the result reliable. The five individuals of the other series strengthen the conclusion that in *Cassiopea* a second regeneration of the margin occurs more rapidly than the first.

3 *The Effect of Rhythmical Pulsation of the Disk on the Rate of Regeneration*

I am indebted to Dr. A. G. Mayer for the method of obtaining a rhythmically pulsating disk. The margin with its sense organs and the mouth apparatus is removed and the ventral surface is cut as shown in Figs. 3 and 4. Such a disk remains quiet if undisturbed, but a rhythmical pulsation can be produced by an electrical stimulation at *X*. This pulsation in some cases continues for several days after a single stimulation. In an animal operated on in this way there are two regenerating surfaces, an outer one to replace the sensory margin and an inner one to replace the mouth apparatus. In my experiments a comparison was made between those with pulsating and those without pulsating disks, to see whether pulsation has any effect on the rate of regeneration.

TABLE III

WIDTH OF REGENERATED INNER MARGIN		WIDTH OF REGENERATED OUTER MARGIN		Duration of regeneration in days	Duration of pul- sation in stimu- lated individuals
Stimulated individual	Unstimulated individual	Stimulated individual	Unstimulated individual		
1.0	2.5	.5	1.0	5	3 days
1.6	2.3	.5	1.0	5	4½ days
1.3	6.0	.6	.7	5	no pulsations
6.0	1.8	1.0	.6	5	2 days
1.6	2.8	.5	1.0	4	8-16 hours
2.8	2.8	1.0	1.0	4	8-16 hours
2.4		1.0		4	few minutes
1.2		.5		4	no pulsations

Two factors must be considered as entering into the result of the present experiment, first the stimulation of the animal as a result of the electrical shock, and second the pulsation resulting from this. It was very difficult to get pulsation that would continue for a considerable period of time. In all cases it stopped before the completion of the experiment. The data are given in Table III, which shows that the effect of the stimulation and pulsation is inhibitory on the whole. In the table, as in the

experiments, the individuals, except the last two are grouped in pairs, one member of each pair being stimulated, and the other one unstimulated.

In four of the six pairs of cases the unstimulated individuals show a greater amount of regeneration than the stimulated ones. In one the two are equal and in the sixth the stimulated is greater than the unstimulated.

DISCUSSION

1 *The Relation of the Degree of Injury to the Rate of Regeneration*

The results obtained in *Cassiopea* agree with the general rule I have found to hold true in the arms of *Ophioglypha lacertosa* and the chelæ of *Cambarus propinquus* and which Ellis finds in the legs of *Mancasellus macrourus*. The rate of regeneration of a removed appendage is determined not only by the character and position of the cut surface, but also by the character and extent of other injuries received at the same time. The rate increases with added injuries to other parts of the body up to an optimum which represents the amount of injury most favorable for regeneration. Beyond this point added injury causes a decline in the rate of regeneration. In the case of the arms of *Cassiopea* the optimum comes when six of the eight arms are removed.

2 *The Relation of Successive Injury to the Rate of Regeneration*

Careful investigations of the rate of regeneration after successive injury are rare in the literature. The general statement is, however, frequently made that the rate and character of regeneration are unaffected by successive injury within very wide limits. I have been able to find descriptions of three cases which do not agree with this statement.

Vanlair finds that the sciatic nerve of the dog regenerates more rapidly after the second than after the first removal.

Driesch finds in *Tubularia* that the development of the aboral hydranth is more rapid after a second than after a first removal. He also makes out an interesting case of effect of successive removal on the character of regeneration in *Antennularia*. The free basal end of the stem of this hydroid, after a first removal, develops

stolons alone. After a second removal it develops one or more slender stems as well as the stolons, after a third removal two or three strong stems and only one or a few slender stolons, and finally after a fourth removal no stolons at all, the whole growth consisting of one or two very stout stems.

To further test the question of the influence of successive injury upon regeneration, I made a study of the problem in the chelæ of the Gulf-weed crab, *Portunus Sayi*, in the Scyphomedusan *Cassiopea xamachana* and in several other forms. The report on the first of these is now in press, and the data show that while the second regeneration is greater than the first, nevertheless, when the age factor is eliminated the two are exactly alike. In *Cassiopea*, however, in which the age factor is also eliminated, the margin shows in every case a greater rate of regeneration after the second than after the first removal. The material on several other forms is now being worked up.

3 *The Relation of Functional Activity to the Rate of Regeneration*

In view of recent discussions concerning the relation of form regulation to functional activity a comparison of pulsating with non-pulsating disks of *Cassiopea* is of special interest. Contrary to the general view that functional activity is an aid in effecting form regulation, it was found that pulsating individuals, with two exceptions, showed a slower rate of regeneration than non-pulsating ones. The result indicates that there is need of further investigation along this line before general conclusions are made.

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STUDIES IN ADAPTATION

I THE SENSE OF SIGHT IN SPIDERS

BY

ALEXANDER PETRUNKEVITCH, Ph.D.

WITH SIX PLATES

Whether the organization of an animal is the result of continuous adaptation to the conditions of life, or whether the animal adapts itself as best it may to the use of organs in the possession of which it has been put by nature, an impartial observer sees everywhere the remarkable balance between structure and function. The few apparent incongruities which we meet with in all groups of the animal and vegetal kingdoms, only serve to confirm the rule, since they are due largely to our lack of knowledge of the conditions under which such organs exist to their best advantage or of the rôle which they played in the phylogenetic development of the species. But however this may be, we should expect to find, and there surely must be a marked difference between the origin of organs by means of which the animal communicates actively with the surrounding world and that of such organs or structures as lie entirely outside of the personal activity or control of the animal. It is very probable that many, perhaps the majority of arthropods are entirely unaware of the presence on their bodies of so-called decorative structures, designs or colors and whether a male possessing a slight variation in such a structure, design or color, will or will not be preferred by a female, is to my mind entirely a matter of chance. In this connection I can only confirm the observation of earlier naturalists that females often choose defective males in the presence of other in every respect perfect ones. I have caught in Europe a pair of large beetles (*Prionus coriarius*), in coitu in the presence of two other perfect males, when the male in question had

one eliter and one leg malformed and showed other minor defects. Upon investigation the male proved to have normal spermatozoa and the female laid an extraordinarily large number of eggs. Similar cases are common among all insects as well as other animals. I have made the same observation in the beetle, *Lucanus cervus*, the males of which have the beautiful horns on their heads. Of the beautiful moth, *Samia cecropia*, over a hundred of which I allowed to develop in a cage for other experiments, many couples with entirely defective and malformed wings were found in coitu. Among orb-weaving spiders are several species in which during the mating period the female allows several males to remain in her net, of course at a good distance from her, and it is a common occurrence for defective males to be accepted by the female while perfect ones will be chased away for their dear life or will even fall victims to the voracity of the much stronger female. Protective structure or coloration belongs to the same group of phenomena and here a great number of cases might be recorded where an animal remains entirely unaware of the protection afforded by its form and color if in the proper surroundings and of the danger of disregarding this. I have seen butterflies that would be protected by the leaf-like coloration of their wings if they should sit motionless, instantly caught on the wing by large dragonflies, and in the West Indies I could observe regularly a hemipteron of the group *Emesidæ*, which would resemble a dead twig if in an appropriate position, swinging to and fro on the four hind legs in the center of a large leaf. All this I bring here by no means with the object of denying the existence of adaptation or of the principle of selection itself, which only one who has never observed nature outside of the laboratory can do. But I want to show that in all those instances where the animal itself cannot make use of a new variation or mutation, in the possession of which it has been placed by nature, its advantageous character will be a mere matter of chance, and selection, if it take place, must be greatly retarded in its progress.

Quite different must it be with those structures which the animal is able to put to active use, however unconscious it may be of the advantage it enjoys over other individuals of the same species.

A caterpillar that is able to construct a better protected cocoon than its fellows, an animal that can run faster from danger, a bird of prey that distinguishes its victim at a greater distance, all these will evince their superiority over other individuals of the same species upon the first occasion. Here, then, opportunity is given for a more rapid accumulation of characters and an accelerated selection. Several years ago I studied, together with Dr. von Guaita, although with a different object in view, the stridulating organs of Orthoptera. These organs are undoubtedly not of a protective character as the insects in question are safer when silent. This is sufficiently shown by the fact that they stop their cry instantly upon the approach of danger. The origin of the stridulating organs has therefore to be sought in some advantage which they may afford the sexes during the mating period. That they are a rather late acquisition is evidenced by their late appearance, only with the last moulting, while the rapidity of selection which in this case must still have been retarded by the danger arising from their possession, is apparent from the complete absence of any traces of similar organs previous to the last moulting. The great variability in the number of teeth on the chord or bow of the stridulating apparatus, which permits of difference of pitch in the tones produced by friction, the fact, with other words, that the stridulating organs are in respect to their details, not absolutely fixed structures, seems to be due to this antagonism between the advantage afforded by the organs in the relation of the sexes toward each other and their disadvantage in respect to diminished safety from the aggression of enemies. But how is it with organs in which the advantage arising from their perfecting will be immediately exploited to its fullest extent by the animal possessing them? With this question before me I commenced my research on the sense of sight in spiders. The results of this research which now extends over more than a year, I bring in this paper.

Two factors in the life of spiders have left a deep impression on their organization, the first, that they subsist exclusively by means of prey and the second, that the external sexual organs,

the organs of copulation in the male, are entirely separate from the internal sexual organs. If we may judge of the amount of food required by the quantity of insects captured in the orb-net of a single spider, a quantity that is sometimes appalling, it was necessary for the spiders, in order to satisfy this want, to develop as they indeed have done, either the instinct and the engineering capacity for constructing nets or else the instinct for hunting their prey freely on the ground or on plants. Two different directions are thus given to the development of the whole spider group and we should naturally expect to find differences in the structure of the corresponding organs of sense and of the spinning apparatus. But while in higher animals, as for example, birds of prey, all organs of sense are developed to a remarkable degree of perfection, we cannot say the same for the hunting spiders. It is a matter of common observation that vultures discern their prey from a distance amounting at times to several thousand feet and they are undoubtedly able to scent a carcass hidden among bushes at a considerable if not so great a distance as this. Their ears are sensitive to high and low sounds of a very small amplitude and only the sense of touch which could scarcely be of use to them, is little developed. It is otherwise with the hunting spiders. They possess doubtless, a very fine sense of touch, the whole body being covered with hairs and bristles sensitive to the slightest stimulus. In regard, however, to the organs of hearing and smell we are as yet without definite results of any kind. At any rate these two senses are very little developed. Even the question as to the existence of such organs is to my mind far from being settled. Whether the organs discovered by Dahl and the lyriform organs have anything to do with these senses must be determined by new investigation. I myself have made some experiments on the sense of hearing, thus far without any definite results. I also repeated the experiments of Pritchett on the sense of smell in large lycosids but even to such irritants as formaldehyde, osmic acid and acetic acid, they did not respond so readily or so quickly as to the slightest touch with the end of a silk thread. As the sense of touch is solely protective, there remains only the sense of sight to guide the spiders on their hunting trips. The

splendid experiments of Mr. and Mrs. Peckham on the jumping spiders (*Attidæ*), during the mating period, have clearly demonstrated that the sexes recognize each other by the use of their eyes alone, the male remaining unaware of the presence of a female, nor will he perform the peculiar, characteristic love dance, if his eyes are covered with paint. But is the sense of sight in spiders as sharp as we should be led to expect by comparison with that of birds of prey? This question has not yet been answered with sufficient certainty owing to the difficulty of experimenting. Plateau came to an entirely negative conclusion, asserting that spiders possess an indistinct and very poor vision, being unable to discern objects beyond a distance of from 8 to 10 cm. Forel was of the same opinion, while the more ingenious experiments of the Peckhams leave no doubt that the males of the *Attids* recognize their females at a distance of about 30 cm. and moreover that they distinguish colors. But whether spiders can see beyond this distance and how sharp their vision is, the experiments of the Peckhams also, leave unanswered. In fact it would be impossible to answer this question merely by observing the behavior of spiders during an experiment or in nature. A close anatomico-physiological study is required and only by combining the experiment on the living specimen with its after examination may one reach a satisfactory answer. This answer I hope to have given in the present research.

Keeping in mind all that has been said in the preceding pages, we may conclude that the study of the eyes of spiders and of their sense of sight, as examples of adaptation of the first kind, possesses several advantages as well as certain disadvantages and these we have next to consider. The advantages are:

- 1 That the sense of sight is beyond any doubt the only sense that guides hunting spiders on their hunting excursions and in finding the females during the mating period.

- 2 That the eyes of spiders are organs which are for each species definite in number and position on the cephalothorax.

- 3 That the sense of sight is a sense common to the great majority of lower and higher animals and that some analogizing is therefore not only admissible but may be of great value.

The disadvantages are:

1 That beyond some experiments of Plateau and the Peckhams, nothing definite is known in regard to the acuity of vision in spiders and no method has been brought forward for its study.

2 That the eyes are complicated organs, consisting of a refraction and a perception apparatus, each of which and the separate parts of which may possibly be capable of adaptation and which have therefore to be considered separately.

Let us now begin with a closer study of the ocular group on the cephalothorax of various adult spiders.

THE POSITION OF THE EYES ON THE CEPHALOTHORAX

The number of eyes in the great majority of spiders is eight and the group which they form on the cephalothorax is so characteristic for the different families that it was for a long time used as a systematic character of great value. The group consists usually of two or three rows, more rarely of two or three smaller groups containing three or two eyes each. Of importance for systematics are the length of each row, the distances between the eyes taken in relation to their diameters, the form of the row, whether straight or bent in the middle forward or backward, as well as the shape and to some extent the color of the eyes. Even a superficial observer will notice in addition, that both in the jumping and the ground spiders (*Attidæ* and *Lycosidæ*), two of the eyes on the forehead are larger than the others, especially in the former. But the reason for such a configuration in the eye-group was never sought for and the apparent similarity in the eye-groups of spiders belonging undoubtedly to different families, led to the conclusion that the value of the eye-group as a systematic character had been overestimated.

The position of the eyes on the cephalothorax becomes more comprehensible when we begin to study the directions of their respective axes and the angles that these axes form with the three chief planes of the body. Unluckily we at once meet with great difficulties even though we choose species having perfectly round eyes. The first of these is to ascertain with exactitude the posi-

tion of the two planes intersecting the plane of symmetry of the body. After experimenting for a long time I decided upon the following method. The spider is killed in alcohol and then kept in it for several days. When the muscles have become entirely rigid, so as to allow of no change in the shape of the cephalothorax while drying, the abdomen is severed from the cephalothorax through the petiolus with a sharp pair of scissors. Next the pars labialis, together with the laminæ, the palpi and the chelæ, is carefully removed with forceps. At the same time care must be taken not to tear the chitinous bridge connecting the opposite sides of the cephalothorax immediately behind the chelæ since this would lead to a flattening of the cephalic part and consequent distortion of the true angles. The legs are now carefully removed leaving the coxæ alone in their normal position attached to the sternum. Next a thin line is drawn with chinese ink on a very thin layer of Canada balsam that has been spread with the finger over a small cover-glass. It is best to use rather a thick cover-glass and to draw the line quite across it parallel to two edges. A drop of fish glue is now put in the center of the glass and on this the cephalothorax is placed with the sternum toward the glue and gently pressed until all the coxæ and the sternum are in contact with the glass. The glass now represents one of the two planes intersecting the plane of symmetry at right angles. I shall call it *the horizontal or foundation plane*. It is only approximately parallel to the surface of the earth or to that of any object upon which the spider may be, since the spider is able to raise itself up on either front or hind legs, in this way changing the angle between the foundation plane and the horizon. At the same time it is essential that the plane of symmetry should coincide with the black line on the cover-glass. This is accomplished by using a needle under the microscope at a magnifying power of about twenty diameters. It is now easy to determine the *plane of symmetry* by taking the point midway between the front middle eyes, the central, longitudinal groove of the cephalothorax when it is a species possessing this groove, and the point at an even distance between the two chitinous plates of that part of the petiolus which remains with the cephalothorax after the abdomen has been

severed. The third plane which I call the *vertical or transverse plane* and which intersects the two other planes at right angles, is geometrically determined by these. After the cephalothorax has been prepared in this manner and thoroughly dried in a warm place, the cover-glass upon which it is fixed is laid upon the center of a square glass plate, which is at the same time the center of a circle drawn upon the surface of the plate with radii forming angles each of which measures ten degrees. If we now use an eye-piece having two lines intersecting in the center at 90° , we can readily measure the angles that the eye-axes form with the plane of symmetry. To know how this is done we must remember that the eyes of spiders have each a lens, the outer surface of which forms part of the surface of a sphere. When we look at a lens in the direction of the eye-axis, it appears to us as a circle while if we look at it under an angle of less than 90° , its outer surface appears to us as two curves intersecting each other at two points. If we place the eye in the center of the microscopic field and move the eye-piece until one of its lines falls upon these two points of intersection of the curves of the lens and the other passes through a point midway between them, then the latter line represents the projection of the eye-axis on the horizontal or foundation plane and the angle that it forms with the plane of symmetry can be read directly from the scale. More exact results would of course be obtained by using a goniometer ocular such as is made by Zeiss, but even the arrangement I have described here, the ocular with intersecting lines, gives an error of not more than a few (3 to 4) degrees. When the angles have in this way been measured a drawing is made with the aid of an Abbe drawing apparatus of the entire eye-group and the eye-axes are then drawn in, in accordance with the data given by the measurements. We obtain in this way a correct figure of the projection of the eye-group on the horizontal or foundation plane. In order to gain a clear picture of the position of the eyes it is necessary to make two other projections, one on the plane of symmetry and the other on the vertical plane. To accomplish this I put a small drop of beeswax in the center of the circle on the glass plate into which the edge of the cover-glass holding the cephalothorax is pressed. This

cover-glass is held in a vertical position with the aid of two straight angles which are removed as soon as the wax is hard enough to keep the glass in position. Another method, much simpler and perhaps just as good, consists in adjusting the cover-glass until its upright edge forms a straight line with its own reflection in the slide. The angles are now measured in the same way as before. The drawing is of course made under the same magnification and the upper edge of the glass holding the cephalothorax is likewise drawn. It represents a cross-section through the horizontal or foundation plane.

To make a drawing of the projection on the vertical plane one proceeds in the same manner with that difference that the cover-glass carrying the cephalothorax is placed on its other edge.

Two facts become immediately apparent upon comparing the drawings made in this way, of eyes of spiders belonging to different families. First, that there is not a single pair of eyes which are focused upon a single point like, for instance, the eyes of man. On the contrary, *the axes of all eight eyes are so directed as to form divergent angles with each other.* Second, that not only do the positions of the eyes on the cephalothorax of spiders belonging to different families differ from each other, but *the axes of the same eyes in different spiders do not lie in the same direction but form with the three planes of the body, the planes of projection, angles differing considerably from each other but fixed for each species.* To make this clear let us look at the drawings (Figs. 1-9) representing the eyes of three hunting spiders, *Lycosa nidicola*—a common large ground spider of northern America, *Phidippus tripunctatus*—a large jumping spider belonging to the same region and *Heteropoda venatoria*—a cosmopolitan tropical and subtropical spider of very large size, belonging to the family Heteropodidæ and resembling in habitus the crab spiders (Thomisidæ) among which it was wrongly placed by earlier systematists.

Translating the results of these measurements into common language, we may thus describe the positions of the eyes in the three spiders. The anterior middle eyes in the jumping spider, *Phidippus tripunctatus*, are directed forward and a little outward and downward. In *Lycosa nidicola* more outward and consider-

ably upward. In this respect *Heteropoda venatoria* resembles *Lycosa* more than it does *Phidippus*, since its anterior middle eyes are also directed frontward but still more outward and considerably upward although not so much so as in *Lycosa*.

TABLE I

Projection on the horizontal or foundation plane. See Figs. 1, 2 and 3

The angles which the axes of the eyes form with the plane of symmetry. The right side with a plus, the left with a minus sign

		AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>	±	8	21	67	95
<i>Lycosa nidicola</i>	±	12	24	24	94
<i>Heteropoda venatoria</i>	±	32	27	0	109

TABLE II

Projection on the transverse or vertical plane. See Figs. 6, 8 and 9

The angles which the axes form with the plane of symmetry. Signs as before

		AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>	±		?	57	63
<i>Lycosa nidicola</i>	±	?	155	?	56
<i>Heteropoda venatoria</i>	±	?		8	71

TABLE III

Projection on the plane of symmetry. See Figs. 4, 5 and 7

The angles which the axes of the eyes form with the horizontal or foundation plane. Zero in front of the head, 180° at the back. Positive quantities for eyes looking upward, negative for those looking downward.

	AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>	- 2	0	+ < 90	+ > 90
<i>Lycosa nidicola</i>	+ 14	- 18	+ 10	+ > 90
<i>Heteropoda venatoria</i>	+ 8	+ 8	+ 92	

The anterior side eyes in *Phidippus* are so directed that their axes are parallel to the horizontal or foundation plane and turned a little sidewise. In *Lycosa* they are directed a little more outward and at the same time downward. In *Heteropoda* they are directed still more outward but at the same time upward.

The posterior middle eyes in *Phidippus* are directed considerably toward the side and upward. In *Lycosa* they are directed much more frontward and also upward, while in *Heteropoda* they look straight upward. Their axes are in this case almost perpendicular to the horizontal plane.

The posterior side eyes in *Phidippus* are directed sidewise, considerably upward and a little backward. In *Lycosa* they are directed in the same way sidewise and backward but considerably more upward, while in *Heteropoda* they are directed considerably less upward and much more backward.

I studied the positions of the eyes as I have described them here, originally in perfectly ripe females. Six individuals of each species were measured and showed only slight differences in the angles. Since the method employed is not an absolutely exact one, it is difficult to say whether these differences depend upon variation in the position of the eyes or upon defects in the method itself. However, the following facts speak rather for defective measurements than for natural variation. I expected to find that during the post-embryonic development the position of the eyes on the cephalothorax would change with each moulting, approaching more and more nearly to that of the adult female, which should be considered an adaptation to the particular life of the spider. An observation that made this seem still more probable has been made by various scientists at different times, that the eye-group in young spiderlings occupies a relatively larger part of the cephalothorax than it does in the adult spiders. Nevertheless there seemed to me to be occasion for a more thorough study of the eye-group and the eye-axes in spiders of different ages. I had in my possession perfectly ripe females of the three species I have mentioned, several specimens of *Heteropoda* just before the final moulting, others that had to moult twice and three specimens of very small spiders that had still to moult at least three times before attaining maturity and also a cocoon filled with very young spiderlings. I kept several females of *Lycosa nidicola* for a time in large glass jars and preserved both mother and the spiderlings which were in part taken from the cocoon, in part killed while on the back of the mother just as they were about to

leave her. I had, besides, several unripe specimens of unknown age. I had also *Phidippus* in the same stages as *Lycosa*. It is not difficult to obtain these since the female of this species make a tent of web in which she lays the eggs, afterward guarding the young ones for a considerable time. I caught in addition several females of *Pardosa nigropalpis* with young ones on the back.

The method employed is as follows: Instead of measuring each eye and the distances between the eight eyes in each spider, I make with the aid of the Abbe apparatus a drawing of the entire eye-group of an adult female. Leaving the drawing in the same position on the drawing table, I remove the adult female and substitute a younger one. I then try different objectives and oculars until the image on the paper of the eye-group of the younger spider is of the same size as the drawing of the adult. A sheet of clean paper is now put in place of the one with the drawing and the new drawing is made. In this way drawings are obtained of all stages. The angles of the eye-axes are next measured and the axes drawn in on the corresponding figures. In all cases, beginning with the young spider at the time when it is ready to leave the mother in order to commence its own, independent existence and ending with the mother herself, the configuration of the eye-group and the angles of the axes proved to be the same and the drawings made of them on paper, when superposed and examined against the light, coincide absolutely. But this does not apply to the youngest spiderlings, those taken directly out of the cocoon. Although in such spiderlings the eye-group is in general very nearly the same as in the adults, careful measurements show differences which, while not appreciable to the unaided human eye, are nevertheless of great importance. I give these measurements here (Table IV) but shall discuss them farther on when examination of the fields of vision will reveal more clearly their significance. It is unfortunately still more difficult to measure the angles in such spiders than it is in older ones so I give only figures of which I am certain.

If we compare these tables with those for the adult females we shall at once notice the following differences. In the youngest spiderlings of *Phidippus* and *Lycosa* the anterior middle eyes

are directed a little more outward and in *Phidippus* a little more downward also, than the same eyes in the adult. In *Heteropoda*, on the contrary, the anterior middle eyes of the spiderling are directed much more frontward than in the adult.

The anterior side eyes in *Phidippus* and *Lycosa* are directed much more toward the side than in the adult, in *Heteropoda* much more toward the front. The angles of projection on the two

TABLE IV

SHOWING THE ANGLES THAT THE AXES OF THE EYES FORM WITH THE THREE PLANES OF THE BODY IN VERY YOUNG SPIDERLINGS

a Projection on the horizontal or foundation plane. Compare Figs. 4, 5 and 7

		AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>	±	10	30	?	88
<i>Lycosa nidicola</i>	±	?	30	25	85
<i>Heteropoda venatoria</i>	±	11	22	18	60

b Projection on the transverse or vertical plane. Compare Fig. 9

		AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>	±	?	?	?	?
<i>Lycosa nidicola</i>	±	?	?	?	68
<i>Heteropoda venatoria</i>	±	?	?	?	?

c Projection on the plane of symmetry. Compare Figs. 4, 5 and 7

		AME	ASE	PME	PSE
<i>Phidippus tripunctatus</i>		-5	0	?	?
<i>Lycosa nidicola</i>		?	?	+ 30	?
<i>Heteropoda venatoria</i>		?	?	+ 87	?

other planes could not be ascertained for these eyes. It is impossible to study the posterior middle eyes in the young spiderlings of *Phidippus* at all, on account of their extreme minuteness. In *Lycosa* the projections of the axes of these eyes on the horizontal plane is approximately the same as in the adult but their projection on the plane of symmetry shows that they are directed upward at an angle about three times as great as in the adult.

In Heteropoda spiderlings they are directed somewhat sidewise and nearly upward but more toward the front, while in the adult they are directed straight upward and a little backward.

The posterior side eyes in the spiderlings of all three species, especially in Heteropoda, are directed a little frontward instead of backward and in *Lycosa* less upward, also, than in the adult.

The relative sizes of the eyes and of the distances between them are also different for spiderling and adult. Tables V, VI, VII and VIII may serve to illustrate this.

TABLE V
Lycosa nidicola. Diameter of eyes in millimeters

	AME	ASE	PME	PSE
Mother.....	0.361	0.279	0.689	0.541
Spiderling, ready to leave mother.....	0.074	0.057	0.148	0.115
Spiderling, taken out of a cocoon.....	0.049	0.038	0.115	0.115

Or in proportion to the anterior middle eyes which we take as unit of comparison

	AME	ASE	PME	PSE
Mother.....	1	0.77	1.90	1.50
Spiderling, ready to leave mother.....	1	0.77	2.00	1.55
Spiderling, taken out of a cocoon.....	1	0.77	2.30	2.30

TABLE VI
Heteropoda venatoria. Diameter of eyes in millimeters

	Length of cephalo- thorax	AME	ASE	PME	PSE
Adult female.....	10.6	0.45	0.75	0.55	0.70
Immature female before last moulting....	6.8	0.28	0.55	0.40	0.50
Two moultings before maturity.....	5.4	0.22	0.43	0.34	0.45

Or in proportion to the anterior middle eyes

	AME	ASE	PME	PSE
Adult female.....	1	1.66	1.22	1.55
Immature female before last moulting.....	1	1.93	1.43	1.78
Two moultings before maturity.....	1	1.95	1.54	2.04

TABLE VII

Pardosa nigropalpis. Diameter of eyes in millimeters

	Length of cephalo- thorax	AME	ASE	PME	PSE
Mother.....	2.8	0.115	0.115	0.328	0.246
Spiderling, ready to leave mother.....	0.85	0.049	0.049	0.115	0.820

Or in proportion to the anterior middle eyes

	AME	ASE	PME	PSE
Mother.....	1	1	2.85	2.13
Spiderling, ready to leave mother.....	1	1	2.34	1.67

TABLE VIII

SHOWING THE DISTANCES BETWEEN THE EYES AND BETWEEN THE EYES AND THE EDGES OF THE CEPHALOTHORAX

Lycosa nidicola. Measurements in millimeters

	Length of cephalo- thorax	Breadth of cepha- lothorax	Distance between outside edges of the PSE	Length of quad- rangle	Distance between the edge of the cephalothorax and the PSE
Adult female.....	8.1	6.0	2.2	1.1	2.2
Spiderling taken out of a cocoon.....	0.984	0.771	0.459	0.197	0.180

Or in proportion to the length of the cephalothorax as a unit

Adult female.....	1	0.74	0.27	0.13	0.27
Spiderling taken out of a cocoon.....	1	0.78	0.46	0.20	0.18

Heteropoda venatoria. Measurements in millimeters

Adult female.....	8.0	8.0	3.2	1.4	1.6
Spiderling taken out of a cocoon.....	1.017	0.902	0.525	0.180	0.131

Or in proportion to the length of the cephalothorax

Adult female.....	1.	1.	0.4	0.17	0.20
Spiderling taken out of a cocoon.....	1.	0.88	0.51	0.17	0.12

By a comparison of all these tables we may now gain considerable light on the question as to what happens to cephalothorax and eye-group during the post-embryonic development. When the spiderling sheds its first skin in the cocoon, the eye-group occupies almost the whole breadth of the cephalothorax which is comparatively very low. The first change, of which I shall speak farther on, is in the directions of the eye-axes. *When the spiderling leaves the cocoon, that is, before the next moulting takes place, the eye-axes have become fixed in the positions which they will occupy during the whole life of the growing and mature spider.* Whatever change takes place from the time the spiderling leaves the cocoon, is only in the relative size of the space on the cephalothorax occupied by the eye-group and to a certain extent, in the relative sizes of the eyes themselves. *The cephalothorax grows more rapidly than the eye-group,* so that the latter occupies with each moulting a relatively smaller part of the cephalothorax. We shall presently see that this also is of advantage to the spider.

THE MAXIMUM *ANGLE AND THE FIELDS OF VISION

While handling under the microscope a dried out cephalothorax from which all organs and muscles had been removed to permit of the study of the endoskeleton, I chanced to notice that the faint images, visible in the eyes, of the trees which grow before my laboratory window, were not of the same size. I very soon found that boiling or even keeping in a cold solution of potassium hydrate so changes the optical property of the eye-lens that it becomes entirely intransparent so that it is necessary to use some other method. I have finally adopted the following one. The spider is killed in strong alcohol from which it is at once removed. The abdomen, all appendices and the sternum are then removed and the organs and muscles filling the cephalothorax are carefully taken out with a forceps. Next the inside of the cephalothorax must be cleaned under water with a soft brush. I use a small camel's hair brush of the kind used for water colors but cut the hairs quite short. With this brush it is possible to remove all the remaining muscles as well as the vitreous bodies of the

eyes. Care must be taken, however, not to remove the black pigment ring surrounding each lens on the inside surface of the cephalothorax and forming a sort of iris, as this would make a difference in the measurements. Even in spiders that have been kept for a long time in alcohol the lenses are often still so transparent that one may see the images formed by them, but such lenses are yellow and the images rather poor. If on the contrary the eyes are prepared in the manner just described, it is scarcely possible to give an idea of the beauty of the little images. The lenses are then entirely colorless and transparent and the images render correctly color and line.

For the study of the fields of vision, each eye together with a little of the surrounding chitin must now be cut out of the cephalothorax. The lens is then placed with its inner surface on a small drop of liquid on a slide and in a hanging position examined under the microscope through the slide. By this means three advantages are gained. First, the object examined sends its rays through the lens in the normal direction so that the eye of the examiner is substituted for the retina of the spider; second, the observer looks in the direction of the eye-axis or at least very nearly in that direction; and third, the outside surface of the lens remains dry, limited by the air alone, as is the case with the living spider. More difficulty is presented by the fact that the refraction-coefficient of the vitreous body is not known. However it is sometimes possible to prepare an eye fresh with the vitreous body in its natural position and the retina cut off with the aid of a razor. We are then able to measure the image of a scale at a given distance. But since the vitreous body coagulates too rapidly to be used in the study of the maximal and minimal angles of vision, we have to use in its stead a drop of water and also of some liquid possessing a *higher* refraction-coefficient than the vitreous body. With the latter we obtain a somewhat larger image than in reality and may *overestimate* the acuity of vision. Such a liquid I found in a mixture of equal parts of pure glycerine and egg albumen. The results obtained by the use of water we may then employ for control, to guard us against the opposite extreme of an *underestimation* of the acuity of vision.

The proportion between the two media is according to my measurements as 4 : 5, *i. e.*, that an image will occupy four divisions of a scale if water is used as the medium of suspension as against five divisions when glycerin-albumen is used.

The microscope also must be arranged in a special manner. The diaphragms with the mirror and the Abbe lens must be removed. The instrument is then placed on a high box open to the window, with a long slit occupying the whole space between the legs of the stand. Next a scale in the form of a cross with right angles is drawn on bristol board. Each arm of the cross is two centimeters wide and consists of alternating black squares like those on a checker-board. For the sake of convenience as well as to avoid error, the numbers are written in each white square in roman numerals in one direction and arabic in the other. This cardboard is now placed under the microscope so that the distance between it and the spider's eye is exactly 10 cm. Excessive light around the eye is excluded by means of a small diaphragm or a black paper with a small round hole arranged so that the spider's eye hangs directly in the middle of the hole. Of course any objective with small magnifying power may be used. As for myself I either use the a^* or the A achromatic system of Zeiss and the compensation ocular 6 with the ocular micrometer. This micrometer is adapted to apochromats but may just as well be used with achromats if one ascertains the size of each division. In my instrument each division of the micrometer with the A objective corresponds to 0.0164 mm., while the correction of the a^* lens makes possible a magnification where each division will correspond to 0.1 mm. The light reflected from the white bristol board on which the scale is made, is sufficient to give a perfect image of the scale in the microscope.

The scale is so placed that the center of the cross falls exactly on the axis of the spider's eye. I found that the eyes of the hunting spiders are quite round and that the maximum angle of vision is therefore the same in each direction, *i. e.*, the limit of the field of vision in such eyes is a circle representing the circumference of the base of a cone. In order to find the maximum angle in each case, there remains only to read on the image of the scale

in the eye, how many centimeters are visible. Since the distance between the spider's eye and the scale equals 10 cm. a simple calculation will give the value of the angle in question, or it is still simpler and entirely sufficient for our purpose, to draw on paper an isosceles triangle, the base of which must be as many centimeters long as are visible on the image of the scale in the eye and its height 10 cm. The angle can now be directly measured. When the angles have been measured for all the eyes, they are represented on the drawings in each projection, showing the field covered by each eye. Optically the angle depends upon the curvature of the lens and the refractive coefficient of the substance of which it consists. But what is of interest for us here is the general fact that the larger the spider's eye, the smaller, as a rule, is its field of vision.

If we compare the drawings of corresponding projections in different spiders after the maximum angles of vision have been introduced, we cannot fail to recognize the remarkable relation between the particular life of the spider and the position of its eyes. In order to make this clear I must state here that which I shall prove farther on, that the larger the spider's eye, the sharper its vision or power of distinction. Let us begin with an examination of the projection on the horizontal or foundation plane (Fig. 1). We see that the largest eyes in *Phidippus* are the anterior middle ones covering a field of 40° each or both together about 55° , owing to the fact that their axes are a little divergent. Each of the anterior side eyes also covers a field of 40° or both together 83° , *i. e.*, more than the entire field covered by the AME.¹ But the ASE are considerably smaller than the AME. The minute eyes of the second row, the posterior middle eyes, cover a field of 62° and the PSE one of 48° or the whole eye-group covers about 240° of the horizon. The projections on the other two planes (Figs. 4 and 6) show in addition that the PME and the PSE guard chiefly the sides of the spiders, leaving about 52° in the vertical plane and more than 80° at the back on the dorsum, entirely unguarded. This is the only direction from which the

¹ These are abbreviations commonly used by arachnologists. AME stands for anterior middle eyes; ASE for anterior side eyes; PME for posterior middle eyes, and PSE for posterior side eyes.

jumping spider can be taken unawares by an attacking enemy. We know besides from the behavior of the spider that whenever an insect or anything else approaches it from the side, it immediately turns toward the intruder as though with the desire to see it better by using its front eyes.

Lycosa nidicola is a spider that lives on the ground under stones, making excursions in the grass. Its manner of walking like that of all ground spiders, is distinctly straight forward and we find that the largest eyes, the posterior middle eyes, are so situated as to guard the front of the animal. In the projection on the horizontal (Fig. 2) plane they together cover a field of 48° , *i. e.*, considerably less than the four eyes of the front row, which cover all together a field of 77° . Between the eyes of the second and those of the third row there is an unprotected area of about 7° , or remembering that the drawing is considerably enlarged, we may say that an object 1 cm. sq. will be invisible within the space of these 7° as soon as it is farther than 8 cm. from the spider. The presence within this area of a spider of the same species could be already noticed at a distance of about 25 cm., quite sufficient to protect against sudden onslaught. The posterior side eyes which are second in size, guard the spider at the sides and back. Thus the entire eye-group covers about 253° of the horizon and leaves unprotected a space on top and at the back.

In *Heteropoda* (Fig. 3) the largest eyes are the posterior side eyes. The four front eyes cover a field of 145° . Between them and the posterior side eyes there is an unprotected area similar to that in *Lycosa*, of about 10° . Or since an adult of *Heteropoda* covers with extended legs about 8 to 10 cm. in each direction, a spider of the same species approaching it within this unprotected area, would become visible at a distance of about 40 cm. The sides are therefore very well guarded especially when we consider that the largest eyes are used in their protection. The eyes of the front row together with the posterior side eyes cover with the interruption mentioned, fully 267° . The dorsal surface of this spider is extraordinarily well protected as compared with the two preceding spiders. There remains an unprotected field of about

10° in front of the posterior middle eyes and about 55° of unprotected field at the back behind these same eyes. In the projection on the plane of symmetry (Fig. 7) the eyes cover 152° or in the normal position of the spider, on a wall, 125° , as may be readily understood from the drawing. In the vertical plane (Fig. 8) the eyes of *Heteropoda* cover a field of 193° . This spider lives in buildings where it runs along the walls and ceilings hunting insects and other spiders and it is distinctly crablike in motion. The comparatively large fields of vision in this species are possibly to be accounted for in connection with the habit of the spider to remain quiet during the day and to begin its activity at dusk. But this does not obscure the fact that the sides of this spider are better protected than the front.

It was next necessary to ascertain whether or not the fields of vision vary in spiders of the same species at different ages. With this object in view many spiders were examined, always with the same result, *i. e.*, from the time when the eyes assume their permanent position on the head of the spiderling, the maximal angles of vision and the fields covered by these eyes are the same as in the mature female. As to spiderlings taken directly from the cocoon, I am sorry to say that I was unable to make any observations upon them. They are so small and their chitin so soft that it is impossible to prepare them in the manner described and I have not as yet devised another method. But assuming that their maximal angles of vision are the same, which is indeed very probable, we may readily see the advantage in the changes of direction in the eye-axes as I have described them. A glance at the accompanying drawings will make this clear. To attain their permanent position the axes of the AME in *Phidippus* move upward and inward. This slight upward change makes it possible for an image to be formed of an object on the central part of the retina of an adult on the same plane, a good deal farther away than is possible in the eye of the spiderling. We shall see farther on that the central part of the retina is much more sensitive than the periphery. The change inward tends to the same end as the change upward and the final position of the anterior middle eyes in *Phidippus* allows therefore of a more perfect distinction of

objects in front of the spider. In the same eyes in *Heteropoda* the direction of the axes changes in the opposite sense, *i. e.*, outward and this change serves to bring about a better discernment of objects considerably at the side of the median line, while the same eyes still guard the front sufficiently. In the posterior middle eyes of *Lycosa*, the most sensitive ones in this spider, the direction of the axes changes to one more downward and inward thus serving to protect better the front. The direction of the axes in the posterior side eyes of the same spider changes in such a way that the adult eyes look farther backward. In both PME and PSE this change takes place at the expense of the field protected in the young spiderling, which now becomes relatively exposed. And here the advantage of a slower growth of the eye-area as compared with the growth of the rest of the cephalothorax, becomes evident. Indeed if the eye-group should occupy in the adult spider relatively the same portion of the cephalothorax as it does in the youngest spiderling, the unprotected field would become in consequence of the change in the direction of the axes, so large that the presence within it of an object even larger than a spider of the same species, would remain entirely unnoticed. In *Heteropoda* the change in the direction of the axes of the PME is in exactly the opposite sense to that in the same eyes in *Lycosa* and affords more protection to the dorsal surface. At the same time in the axes of the PSE in *Heteropoda*, the change of direction is in the same sense as in *Lycosa* and *Phidippus*. But this change is considerably more marked in *Heteropoda* with the result that in the adult spider the eyes cover fully 267 degrees instead of the (probable) 166° in the spiderling. This advantage cannot be gained however, without the formation of an unprotected field. Again, as in *Lycosa*, this field would have been much larger but for the difference in growth between the eye-group and the cephalothorax.

THE LIMIT OF VISION

If we examine under small magnifying power at once all eight eyes of a cephalothorax, freshly prepared and suspended on a drop of glycerin-albumen as I have described, we shall remark

that the four pairs of eyes form four pairs of images differing from each other in size. As a rule we shall find that the largest eyes form the largest images. The question at once occurs, are all eyes equally sensitive notwithstanding that they form images differing in size, or are the larger eyes more sensitive than the smaller ones? The surest way to find the answer to this question is to determine the minimum angle of vision for each eye. But how are we to do this when we do not even know with sufficient exactitude the distance at which under normal conditions a spider recognizes another of the same species. The experiments of the Peckhams, in spite of their ingenuity, still admit of too great range for error, to be utilized in a study of the normal angle of vision. Of what advantage, then, would be a similar experiment but with some of the eyes blackened with paint? It could serve merely to control another method, a method of comparative morphology. We have to start from the proposition that the physiology of the nervous system is analogous in the other animals and man, a proposition which few are disposed to admit, but here the experience gathered in many fields and from observations made on different animals, comes to our aid, an experience that leaves scarcely any room for doubt that the stimulation of a single nerve-ending transmits to the central nervous system a single sensation only, whether or not the stimulus itself is a simple one or in reality composed of many contemporaneous stimuli. Thus, as is well known, in order to perceive two pin-pricks as two distinct sensations, it is necessary that they should be applied to two separate nerve-endings as otherwise the sensation is that of a single prick and again, when the image of two stars falls on only one cone of the retina of the unaided human eye, the eye perceives but a single star. These are well known facts which justify us in saying that in the spider's eye two rods must be stimulated by light rays in order that the image of two points should be produced. But here the analogy ends. How strong the effect produced and whether the corresponding image in the brain is of the same kind as in man, we cannot know. We cannot know whether a spider sees colors as we do, whether green appears to it in the same way as it does to us, although we do know from the experiments of

the Peckhams that spiders are able to discriminate between colors. Neither can we know whether gradations of light and shade are the same for the spider as for us nor how great the amplitude of the light wave, which would be required to produce the same effects as in us.

Nevertheless we do know that an image is formed in each spider eye; we do know that the four pairs of images differ from each other in size; we do know that the more rods covered by the image the more detail can be perceived by the eye. We may thus work on a fairly safe basis.

Let us first examine the images as they appear under the microscope. When a black square is placed under the microscope so that the axis of the eye is perpendicular to the center of the square, it is not possible to detect any spherical aberration by common means. But if we place the eye so that the black square lies considerably to one side of the eye-axis, the aberration at once becomes appreciable. For the accompanying drawing (Fig. 11) an eye of *Lycosa* was put so that it was a little outside the center of the microscopic field and the axis of the eye formed a more or less sharp angle with the slide, while the black square, each side of which was 5 cm. long but having one side prolonged into a straight black line, the whole made with chinese ink on a plate of milk-white glass, was absolutely parallel to the slide. The Zeiss drawing table was carefully arranged beforehand so that a small square placed under the microscope and drawn with the aid of the Abbe apparatus, gave on paper a perfect square. Then drawings of the image in the spider's eye of the black square were made from different positions of the eye, obtained by revolving the table of the microscope on its axis. It is clear from the drawing that in this case the base and one side of the square are especially distorted. Is the spider's vision then distorted of all things that lie out of the axis of the eye? It is impossible to know but I believe that the spider forms a true idea of objects, first because the distortion in each eye of a pair is in the opposite sense to that in the other eye of the same pair, thus offsetting it, and second, because the retina is not a plane but is of very complicated form differing in different eyes and for different species. Generally speaking we may compare

the retina to a boat or canoe in some eyes and to a deep bag in others. This may be ascertained not only from sections. It is sometimes possible to remove the entire retina intact from the vitreous body whose proximal end fills it out and to examine it in toto under the microscope. The vitreous body also varies in shape in different eyes and is usually considerably elongated in the direction of the eye-axis in those eyes which form the largest images. The vitreous body is especially long in the anterior middle eyes of jumping spiders, *Phidippus tripunctatus*, for example, and is shaped something like a long cone with its base which is concave, toward the lens, its axis being at the same time the axis of a conical hole which extends through its entire length. This hole also is largest at the lens and much smaller at the retina and may be seen in sagittal and cross-sections. It is also sometimes visible in young spiderlings of the jumpers, where it presents a likeness to a pupilla. In life it is probably filled out with a liquid.

Roughly speaking the size of the image is in direct proportion to the size of the eye but measurements show discrepancies which must be due to differences in the curvatures of the lenses. The following table illustrates this.

TABLE IX

Ratios of diameters of eyes and of images to the diameter of the AME and its image. Compare with Fig. 10

		AME	ASE	PME	PSE
Phidippus tripunctatus.....	eye	1	0.595	0.166	0.524
	image	1	0.4285	0.0800	0.3143
Heteropoda venatoria.....	eye	1	1.66	1.22	1.55
	image	1	1.75	1.50	2.00
Lycosa nidicola.....	eye	1	0.77	1.90	1.50
	image	1	0.8	2.266	1.8666

This table shows that while there is a dependence of the size of the image upon the size of the eye, this dependence is not of such a kind as to allow of definite conclusions in regard to the smallest angle of vision from measurements of the eyes and

images in one individual and of the elements of the retina in another. I have therefore applied two other methods. The one consists of choosing two individuals of the same species, having eyes of the same diameter and preparing one for the study of the image, the other for sections through the retina. This is possible and yields good results when one has a large quantity of living spiders. By far the better method, the one which I now use exclusively and upon which the conclusions I have reached in this research are based, consists in preparing the spider in such a way as to obtain from the same individual at the same time the lenses intact for the study of the size of the image and the retina for sectioning. This is perfectly feasible even in young spiderlings although it requires not a little patience and experience. I proceed in the following manner: a spider is killed by a cut across the middle of the cephalothorax so as to allow the fixing liquid to penetrate as rapidly as possible. Thus far I have obtained the best results for the purpose with the picro-formalin mixture of Bouin and my sublimate modification of the Gilson liquid. The cephalothorax is allowed to remain for six hours in the fixing liquid and is then transferred in the usual manner to 70 per cent alcohol. The sternum and the mouth parts are now carefully removed with a fine pair of scissors. Then the cephalothorax is placed in a low dish containing alcohol and held by the side with forceps while a thin and flexible spatula is carefully introduced between hypoderm and chitin. Pushing the spatula slowly forward it is possible to separate the entire chitinous part of the cephalothorax from the underlying hypoderm with all its muscles and organs. The vitreous bodies of the eyes remain with the retinas attached by the optic nerves. These are now separated from the remaining organs, carefully noted to exclude error and placed in separate dishes in paraffin in the usual manner. They are then sectioned with a microtome, either parallel to the eye-axis or perpendicular to it. The sections are depigmented in chlorine gas dissolved in 70 per cent alcohol and after washing stained in Heidenhein's hæmatoxylin. The eye-lenses of the same individual, which have been removed together with the tergum, in the manner described, are now placed in water and carefully cleaned

with the brush on both sides. They are then cut separately out of the cephalothorax and suspended, each first on a drop of water and then on one of glycerin-albumen and the image which each forms of a 10 cm. square at a distance of 10 cm. is measured with the aid of the ocular micrometer. These measurements may then be directly compared with those obtained from the sections through the retina. In measuring the distance between the rods I use the highest power only and count how many microns are occupied by ten rods. This is essential since the distances between the rods are apt to vary a little. Besides, the

TABLE X

An adult Lycosa nidicola, small individual. Average distance between the centers of the two rods in micromillimeters

	In the center	Toward the periphery	At the periphery
AME.....	8	12	15
ASE.....	6	10	12
PME.....	8	12	15
PSE.....	9	16	21

rods are larger and farther apart at the periphery, gradually becoming smaller and lying closer together toward the center. I do not give a drawing of this but Table X affords sufficient demonstration.

It seems to me, in view of the strange shape of the retina, that we may form an idea of the acuity of vision in the spider's eye only from images that cover the central part of the retina alone. In order to diminish the possible error, I measured the image of 10 cm. as mentioned, but divided the result by ten so as to find the size of the image of 1 cm. from 10 cm. distance.

In this way we obtain the following table:

TABLE XI

Phidippus tripunctatus

		AME	ASE	PME	PSE
Size of the image of 1 cm. at 10 cm. distance. Eye suspended on glycerin-albumen.....	"	115	49	10	36
Distances between the centers of rods in the center of the retina.....	"	3	6	4	5

On dividing the size of the image by the distance between the rods, we find that the image of a line 1 cm. long (measured in an eye suspended on a glycerin-albumen drop) occupies respectively 38 rods in AME, 8 rods in ASE, 2 rods in the PME and 7 rods in the PSE. One square centimeter at a distance of 10 cm. gives an image that occupies $38^2 = 1444$ rods in the AME, $8^2 = 64$ rods in the ASE, $2^2 = 4$ rods in the PME and $7^2 = 49$ rods in PSE. With other words the front middle eyes in *Phidippus* are by far the most sensitive, then come the front side eyes, then the eyes of the third row and last, the posterior middle eyes.

In *Lycosa nidicola* the difference in the sizes of the eyes is less than in *Phidippus* and consequently the difference in sensitiveness or power of distinction is less. I examined several specimens. Since in the one used for the measurements which I give here the image of 10 cm. at 10 cm. distance did not coincide with the divisions on the scale on my ocular micrometer, it was necessary, in order to avoid error, to arrange the experiment somewhat differently. The bristol board containing the centimeter scale was brought nearer to or farther from the eye until a number of centimeters not fewer than five, came to occupy a certain number of divisions on the scale. Thus I found that at a distance of 37 cm. the image in the PME of 6 cm. occupies exactly 5 divisions of the micrometer scale. Each division being equal to 0.0164 mm., it follows that the image of 1 cm. would occupy $0.0164 \times \frac{5}{6} = 0.0136$ mm. Since the distance between the centers of the rods in the center of the retina in this specimen equals 13μ , it follows that 1 sq. cm. placed at a distance of 37 cm. from the PME will form an image occupying 4 rods (2×2). The image of 7 cm. placed at a distance of 36 cm. from the posterior side eye, occupies 5 divisions of the scale or the image of 1 sq. cm. equals 0.0117^2 mm. and occupies 4 rods, the distance between the centers of these being 12μ ; but this image does not occupy the rods completely. The image of 9 cm. placed at a distance of 30 cm. from the AME, occupies five divisions of the scale or the image of 1 sq. cm. at 30 cm. distance equals 0.009^2 mm. The distance between the centers of the rods in this eye is 9μ , *i. e.*, the image occupies 4 rods (2×2).

The image of 14 cm. placed at a distance of 27.5 cm. from the ASE corresponds to five divisions of the scale or the image of 1 sq. cm. = 0.006^2 mm. The distance between the centers of the rods in this eye being equal to 6μ , the image will occupy 4 rods (2×2). We have then in *Lycosa* also, four pairs of eyes of different sensitiveness in the following sequence from more to less sensitiveness: PME, PSE, AME, ASE.

Unfortunately I have no material of *Heteropoda venatoria* fixed in the manner described, but from comparison with *Lycosa* and *Phidippus* we should expect to find that in this spider the most sensitive eyes are the posterior side eyes followed by the ASE, PME and AME.

In order to form a clear conception of the acuity of vision of the spider's eye, we shall compare the anterior middle eye of *Phidippus* and the posterior middle eye of *Lycosa*, the two most sensitive eyes, with the average human eye. The distance between the centers of the cones in the yellow spot of a human retina is, according to measurements made by various scientists, somewhere between 4 and 5μ . One square centimeter placed at a distance of 30 cm. occupies somewhere in the neighborhood of $114^2 = 12996$ cones in the human eye, $13^2 = 169$ rods in the AME of *Phidippus* and only $2^2 = 4$ rods in the PME of *Lycosa* (Fig. 12). The difference becomes still more tangible if we find the distance at which 1 sq. cm. will fall on one rod in the spider's eye, with other words, if we ascertain the smallest angle or limit of vision. This may be done either by direct calculation from the data obtained or by measuring the image of a centimeter scale that is gradually moved away from the eye until the image becomes smaller than the distance between the rods. In this way I found that the minimal angle of vision, using glycerin-ablumen for suspension of the eye, equals $8'$ for the AME of *Phidippus* and about $60'$ for the PME of *Lycosa*, while from observation of the double stars it is known that the smallest angle of vision in man equals $1'$. Thus a creeping insect about 1 sq. cm. in size would be perfectly visible to the human eye, even perhaps to the extent of recognizing the species, at a distance of about 3 m., while it would appear merely as an indefinite, tiny moving speck to *Phidippus* and would be entirely beyond the range of vision of *Lycosa*.

These figures certainly show the great superiority of the human eye over even the best eye of spiders. This is especially manifest from the fact that even if we use a drop of Canada balsam with its 1.535 refraction-coefficient, for the suspension of the eye we still find the limit of vision for the AME of *Phidippus* to be 6' and for the PME of *Lycosa* to be 45'. At the same time we must not forget that the minimal angle of vision in the spiders is in inverse proportion to the maximal angle and that with the use of the Canada balsam the measurements would show a reduction in the fields of vision from 40° in the AME of *Phidippus* to about only 30°. On the other hand the measurements made with the use of glycerin-albumen show that a female jumping spider placed at a distance of 30 cm. from its mate, would still give a sharp image covering a sufficient number of rods in the eye of the male to be recognized by him—a result which stands in conformity to the experiments of the Peckhams. Perhaps this relation between field of vision and acuity of vision was responsible for the fact that the eyes of spiders did not attain the acuity of vision of the human eye.

We have already seen that the smaller the diameter of a spider's eye, the smaller the image it forms. We have also seen that this does not apply with exactness to different eyes of the same eye-group but measurements show that for the same eyes in spiders at different ages the ratio holds good as far as it is possible to ascertain with the methods used. The younger the spider the smaller the images in its eyes and the question arises: are the eyes of spiderlings as sensitive as those of adults or does the power of distinction grow with increasing age? Spiderlings that are ready to leave the mother can be prepared as I prepared the adult spiders and image and retina may be studied in the very same eye. But this cannot be done with spiderlings taken out of the cocoon so we may base our conclusions on the admission only, that the ratio between diameter of eye and image holds good for these spiderlings also. This is indeed probable since I have proved that such a ratio exists in all ages beginning with the oldest spiderlings. In every case I examined for comparison purposely the mother spider with her young ones, to avoid errors

arising from possible variations hereditarily fixed through several generations. I scarcely need to add that several mothers and many young ones of the same species were examined.

The distance between the centers of the rods in all eyes of the spiderling is smaller than the corresponding distance in the eyes of the adult. Thus in the PME of an adult *Lycosa nidicola* the distance between the rods in the center of the retina was found to be 8μ while the corresponding distance in the PME of the young is only 2.5μ . The diameters of these eyes are respectively 689μ and 148μ . Or the proportion between the diameters is $\frac{689}{148} = 4.65$, while the proportion between the rods is only $= 3.2$. With other words, the image of the same object will cover a smaller number of rods in the eye of the spiderling than in that of the adult, say $6 \times 6 = 36$ rods in the former and $9 \times 9 = 81$ rods in the latter. The same proportion holds for all eyes of *Lycosa* but when I compared the images in the eyes of this adult female with those of her young ones that had been allowed to remain on her back for several days longer and were beginning to leave her, I found that this ratio already falls to $4 : 3.5$ and becomes $1 : 1$ after the next moulting. *Pardosa nigropalpis* showed the same conditions and we should expect the same to be true of other spiders, too.

CONCLUSIONS

In drawing conclusions from the facts described, I am well aware that the methods employed in this research are far from perfect. It is possible that the angles of the eye-axes will with time be measured more exactly, that the figures given for the maximal angles of vision will be a little altered in the one or the other sense, that the minimal angle or limit of vision will be found to be one minute larger or smaller, but in one respect the method employed gives facts that cannot be disputed; I mean that the error, whether large or small, is the same for the entire series of the same kind, so that the relation or ratio will remain unchanged. We cannot be sure that the maximum angle of vision in the AME of *Phidippus* is exactly 40° or that its smallest angle of vision is precisely $8'$, but we may be sure that the larger eyes of an individual

are more sensitive than the smaller ones of the same individual; we may be sure that the eyes of the young spiderling are less sensitive than the same eyes in the adult; we may be sure of the proportional sensitiveness of the eyes; we may be sure that changes take place in the directions of the eye-axes and of the direction of these changes as well as of the time when they take place; and all this goes to show unmistakably the existence of a perfect balance between function and structure in the eyes as well as a remarkable degree and an extraordinary rapidity of adaptation. Thus when the spiderling first begins to lead an independent life, it finds in its eyes an organ already sufficiently perfect to be relied upon in the struggle for existence.

The changes in the angles of the eye-axes may well be looked upon as an adaptation. I have also tried to show that the slower growth of the eye-group as compared with that of the cephalothorax, is to be considered advantageous. The way in which these changes came about is also well understandable but when we ask ourselves in what way in the phylogeny was the increasing acuity of vision accomplished, we have to depend for the answer upon theoretical considerations. We know that in the individual this increasing acuity of vision is brought about as a consequence of the slower growth of the retina elements compared with that of the lens. Is it then a retarding influence of which nature took advantage in order to accomplish its own end, that was responsible or was it a possible capacity on the part of the lens to grow more rapidly than the retina? Or were perhaps both factors at work? And at what time in the phylogenetic development did the eyes differentiate into smaller and larger and why did they not all reach the same degree of perfection? What was it that arrested the progress toward perfection or is further perfection possible and still in process of attainment? All these are questions before which we stand without answer as before a door behind which treasures lie concealed. To find the key to that door would mean to understand adaptation. For a long time science remained satisfied with the explanation afforded by the principle of selection and the attempt was made to apply this principle to all the phenomena of organized life. But the very ease with which it answers

the most intricate questions proves to be its weak point and it no longer suffices. How indeed, for example, can we accept the explanation given for the existence of the black shield at the base of the bill of the white swan, as a protection against the blinding light reflected from the surface of the water, when another water bird of the same fauna—*Fulica atra*—has black plumage and a snow white shield? The difficulty is gotten round by explaining the latter case from the point of view of sexual selection or in some other way. The number of similar cases could be multiplied indefinitely. It is clear that if we desire to hold to the principle it will be necessary to show step by step the progress of adaptation in indisputable cases. Of this nature are variations of which the animal itself can immediately make use to its own advantage over its rivals. While the origin of the variations themselves is a field for other research I hope to have shown in the present study of the sense of sight in spiders, the probable course of adaptation in the survival of the fittest.

Short Hills, New Jersey

August 11, 1907

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EXPLANATION OF THE DIAGRAMS

In the Figs. 1, 2, 3, 6 and 8 the axes of the eyes are represented by dotted lines, the maximal angles of vision and the plane of symmetry by common black lines. In the Figs. 4, 5 and 7 the heavy black line represents the horizontal plane for the upper part of the figures and the plane of symmetry for the lower part of the same figures. The axes of the eyes and the maximal angles of vision in these figures are represented by common black lines for the mature spider and by heavy dotted lines for the young spiderling.

PLATE I

Fig. 1. *Phidippus tripunctatus*, adult female. Projection of the eye-group on the horizontal plane showing the axes of the eyes and the maximal angle of vision for each eye.

Fig. 2. *Lycosa nidicola*, adult female. Same projection as in the preceding figure.

Fig. 1

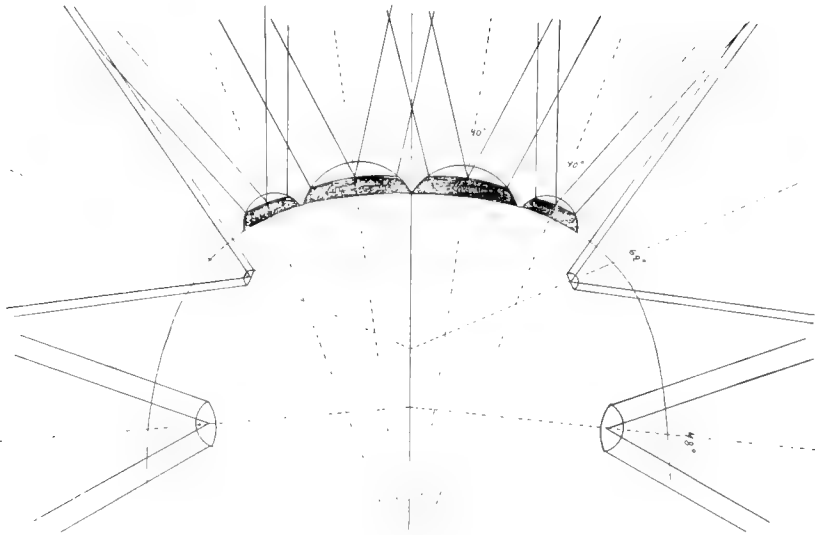


Fig. 2

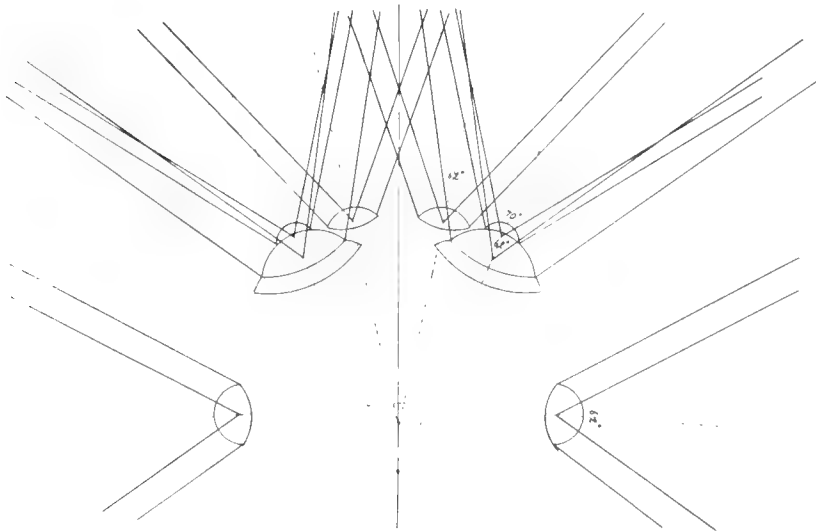


PLATE II

Fig. 3. *Heteropoda venatoria*, adult female. Same projection as in the two preceding figures.

Fig. 4. *Phidippus tripunctatus*, same adult female as in Fig. 1. The upper part of the figure shows the projection of the eye-group on the plane of symmetry while the lower part represents the projection of the left half of the eye-group on the horizontal plane. In the same figure the axes and the maximal angles of vision of the spiderling are indicated by heavy dotted lines and the direction in which the change from the spiderling to the adult takes place is shown by the arrows.

Fig. 3

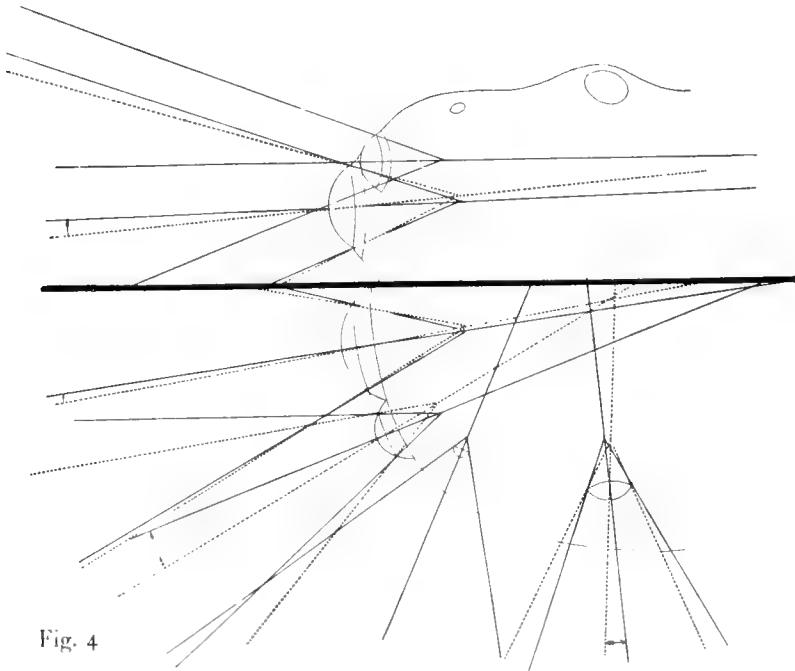
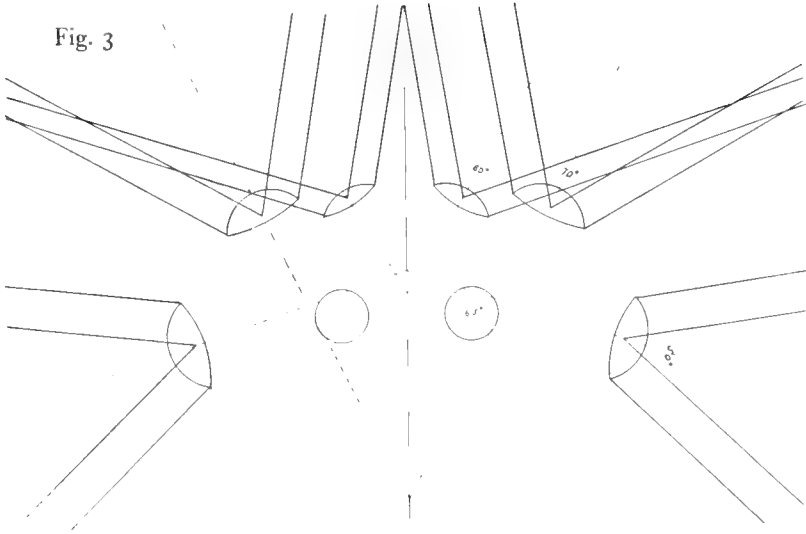


Fig. 4

PLATE III

Fig. 5. *Lycosa nidicola*, same individual as in Fig. 2. Same projections as in Fig. 4.

Fig. 6. *Phidippus tripunctatus*, same individual as in Figs. 1 and 4. Projection of the eye-group on the transverse or vertical plane. The heavy line HH' represents the horizontal plane.

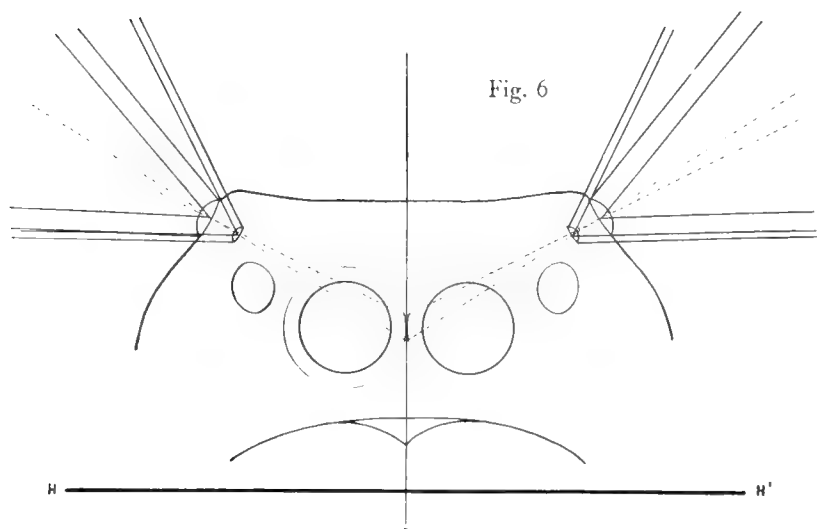
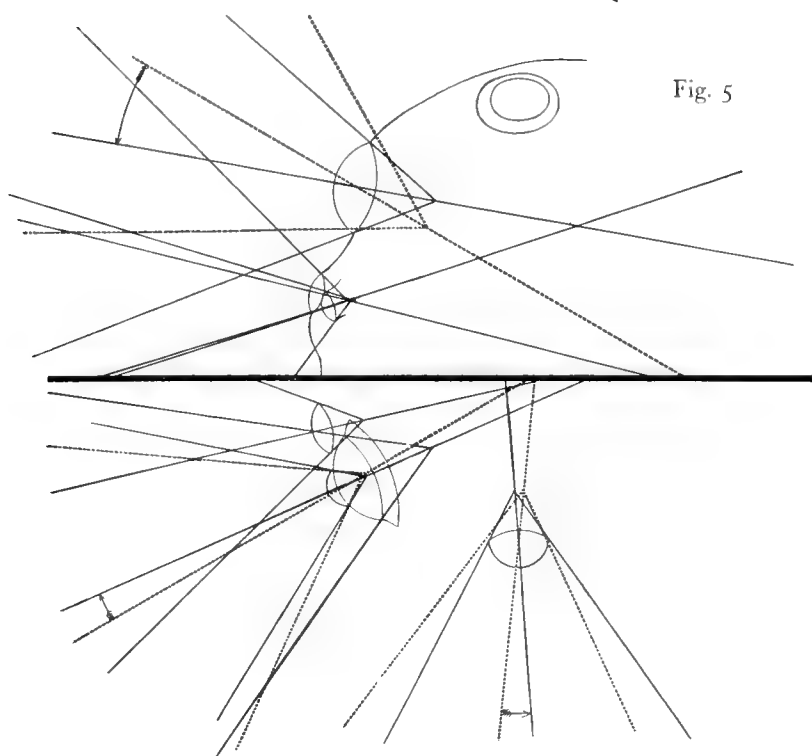


PLATE IV

Fig. 7. *Heteropoda venatoria*, same individual as in Fig. 3. Same projections as in Fig. 4.

Fig. 8. *Heteropoda venatoria*, same individual. Same projection as in Fig. 6.

Fig. 7

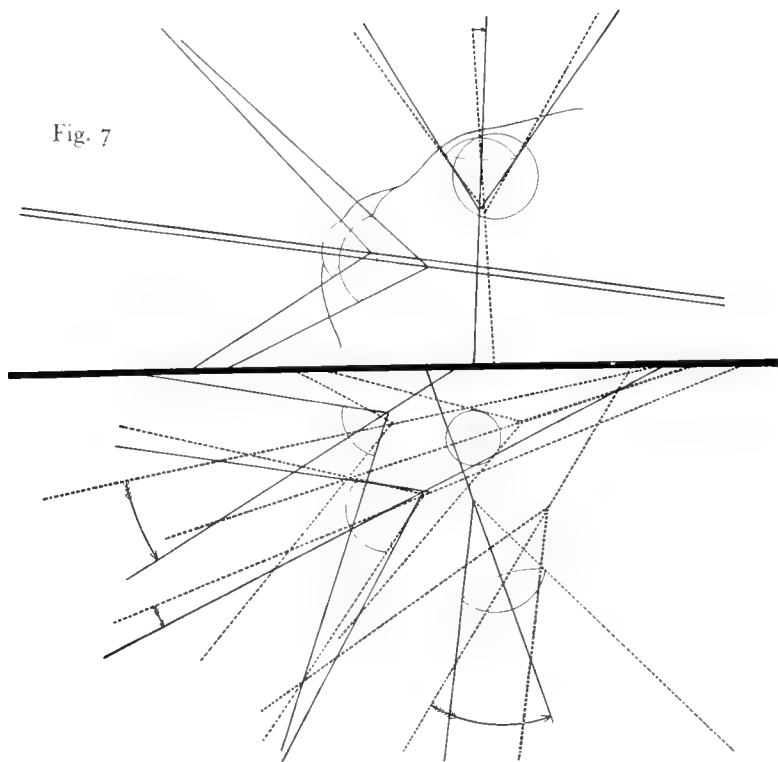


Fig. 8

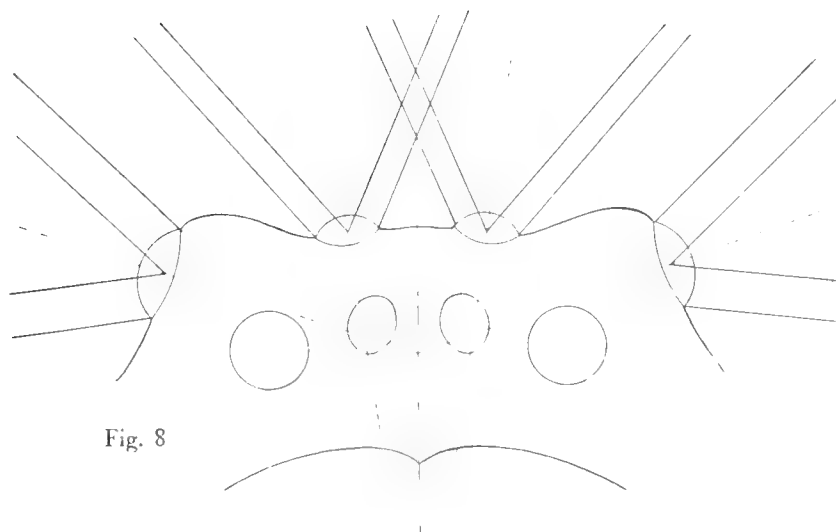


PLATE V

Fig. 9. *Lycosa nidicola*, same individual as in Figs. 2 and 5. Projection on the transverse plane. The arrow indicates the direction in which the change in the position of the axis of the posterior side eyes took place. The axes of the eyes in the mature spider are represented by light interrupted lines, the maximal angles of vision by common straight lines, while the axes and the maximal angles of vision for the spiderling are represented by heavy dotted lines.

Fig. 10. This figure shows the respective size of the image on the retina formed by a black square placed at the same distance from each eye in the three species of hunting spiders. If compared with Figs. 1, 2 and 3 this figure shows also that the larger eyes form the larger images.

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Fig. 9

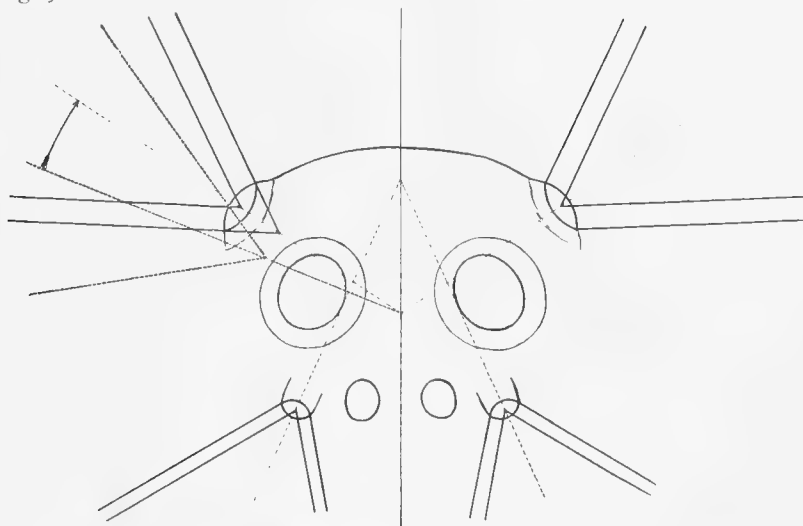


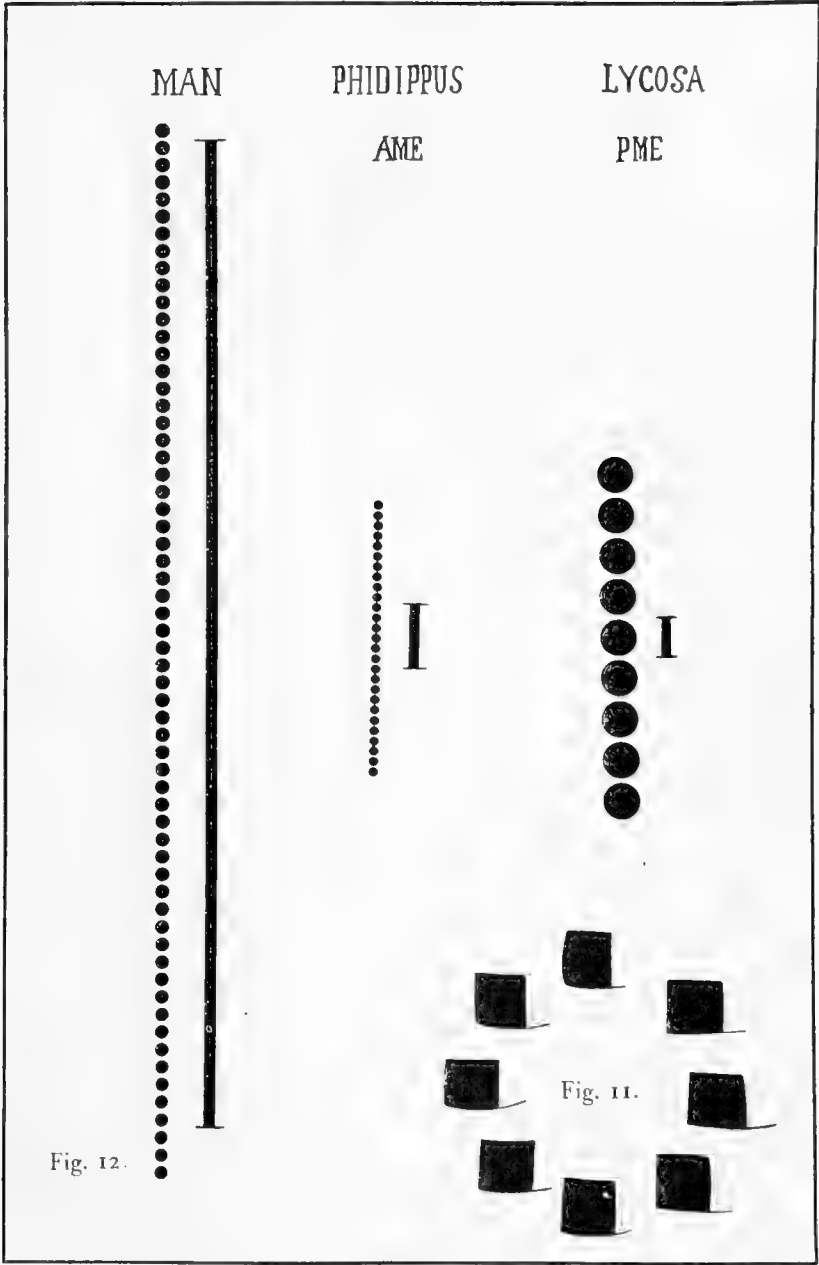
Fig. 10.

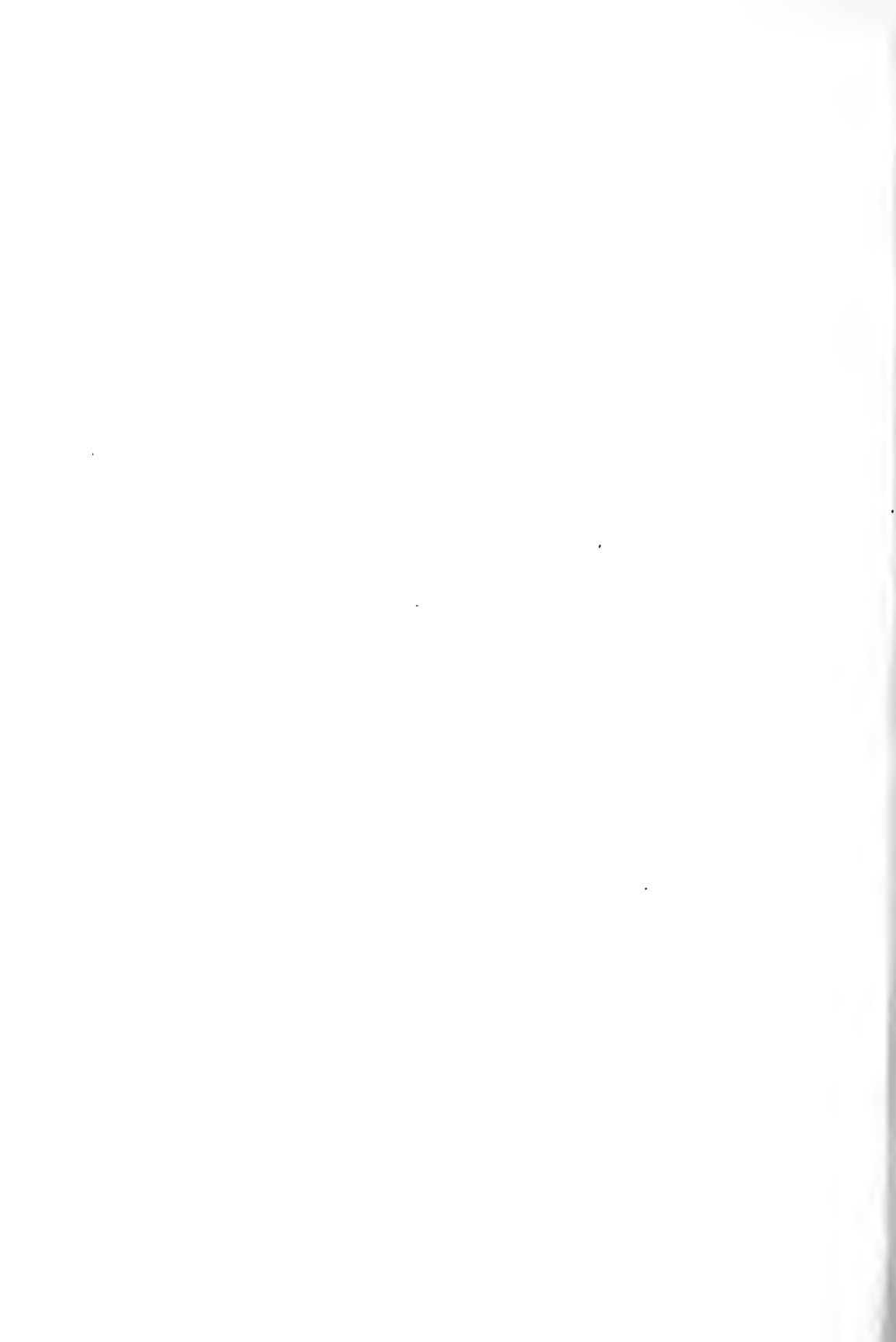
	AME	ASE	PME	PSE
<i>Phidippus</i> <i>tripunctatus</i> (AME=1.) $AME = 1$ $ASE = 0.4885$ $PME = 0.08$ $PSE = 0.3123$				
<i>Lycosa</i> <i>nidicola</i> (AME=0.74) $AME = 1$ $ASE = 0.8$ $PME = 2.247$ $PSE = 1.847$				
<i>Heteropoda</i> <i>venatoria</i> (AME=0.26) $AME = 1$ $ASE = 1.75$ $PME = 1.50$ $PSE = 2.00$				

PLATE VI

Fig. 11. Aberration in the spider's eye. The figure represents the images formed by an eye from a perfect square which was placed in eight different places near the periphery of the field of vision. When a square is placed in the eye-axis its image is free from aberration.

Fig. 12. This figure represents the comparative acuity of vision in man, and in two hunting spiders. The posterior middle eyes possess the greatest acuity of vision in *Lycosa nidicola*, and the anterior middle eyes possess the greatest acuity of vision in *Phidippus tripunctatus*. The black discs represent a row of rods in the retina of *Phidippus* and *Lycosa*, and of cones in the yellow spot of man, all equally magnified. The heavy black lines represent the length of the images formed by the same object placed at the same distance from each of the three eyes. In the human eye this image will occupy 57 cones, in the anterior middle eye of *Phidippus* nearly seven rods, while in the posterior middle eye of *Lycosa* the image is only a little longer than the diameter of a rod.





THE PHYSIOLOGY OF THE NERVOUS SYSTEM OF THE RAZOR-SHELL CLAM (*ENSIS DIRECTUS*, CON.)

BY

GILMAN A. DREW

WITH ONE PLATE

The razor-shell clam is a particularly favorable lamellibranch for the study of the functions of the ganglia, because: (1) It is very active and responds rapidly to stimuli. (2) Each ganglion supplies nerves to organs that are so active that one can hardly fail to see movements, even when the stimulation is slight. (3) The animal is so narrow that the shell valves can be wedged apart enough to allow all operations and experiments to be performed without removing the animal from its shell. (4) The ganglia with their commissures, connectives and chief nerves, all lie so superficially they can be seen without cutting the animal more than to separate the fused margins of the mantle lobes and the inner lamellæ of the inner gills, and to expose or cut almost any one of them requires only the cutting of a thin outer covering that cannot cause a mutilation that needs to be taken into account in the results that are obtained.

Before discussing the functions of the different ganglia it is desirable to study the activities of the animal as a whole and to become acquainted with the responses of the various portions of the body when the organs that are subject to external stimuli are stimulated.

The habits of the animal have already been discussed in another paper,¹ but in studying the effect of stimuli it is necessary to know something of the normal life of the animal, and accordingly a brief statement of its habits are desirable here. The animals are best known on mud-flats that are exposed at low tide, but they are

¹ The habits and movements of the Razor-shell Clam, *Ensis directus*, Con. Biol. Bul., vol. xii, no. 3, 1907.

known to occur at moderate depths. Dr. K. Kishinouye writes me that the Japanese "fishermen catch razor-shell clams from the bottom of the sea, ten or more fathoms in depth, by means of slender spears that are weighted at their upper ends and held at the end of a rope." Inasmuch as the catch is made by simply pulling up and dropping the spear and is dependent upon accidentally striking the clams, they must be fairly abundant to make such a method profitable. The animal lives embedded in the mud almost perpendicularly, with the siphon end usually barely protruding above the surface. Occasionally specimens are found, when the mud-flat is bare, with half or more of their shells exposed, but, judging from observations of specimens in aquaria and of other specimens in their native mud-flats that had not been disturbed and were covered with water, I am inclined to believe that this is not a usual position, and is probably assumed as the result of the stimulation of the heat of the sun.

When an animal is disturbed, as by jarring the mud or by stimulating the exposed siphons, it almost instantly disappears into the mud. This is evidently its means of escape from enemies. The burrowing is done by means of a remarkably long, active, cylindrical foot, Figs. 1 and 2, *f*, that can be protruded from the anterior end of the shell to a distance equal to more than one-half of the length of the shell. When fully extended the end of the foot is swelled to form a knob that serves as an anchor for the animal to draw itself into the mud. The fact that the animal disappears so promptly after it is disturbed indicates that the foot is probably kept somewhat extended when the animal is at rest in its usual position.

The margins of the mantle lobes are fused together so that four openings into the mantle chamber are left. Two of these are the openings of the siphons, Fig. 1, *bs* and *cs*, the third is the opening through which the foot is protruded, and the fourth is a small opening about midway on the ventral margin, Fig. 1, *vo*. What function is performed by the last mentioned opening is not clear. With an expanded animal in a dish of sea-water it is easy to demonstrate that a current of water enters this opening. This is to be expected as the opening leads into the branchial chamber, into

which water is constantly passing, and there is no reason why water should not enter this opening as well as the branchial siphon. When the animal is embedded in the mud however, free admission of water through this opening is not to be expected. The opening is surrounded by well developed tentacles that are similar in appearance to those around the siphons and, like them, very sensitive to tactile stimulations. Stimulation of these tentacles always cause the animal to close its shell and usually, this may be the mechanical effect of suddenly closing the shell, the slight protrusion of the foot. The foot is almost immediately retracted into the shell again and remains retracted unless stimulation is continued. When the stimulation is continued the foot is alternately protruded slightly and retracted, and occasionally, when the animal is held anterior end downward, burrowing movements are started.

On each side of the line of fusion of the mantle lobes are very small papillæ that are probably also very sensitive to touch. The whole region is very sensitive but whether sensation is more acute on the papillæ than on the general surface was not determined. Posteriorly, from the ventral opening to the branchial siphon, the fused mantle margins are very thick and muscular. Anteriorly, to the opening through which the foot is protruded, the margins are loosely attached by their epithelial cells. The extensive fusion of the margins of the mantle keeps mud out of the mantle chamber during burrowing, and forms a device for expelling strong jets of water.

Around the opening through which the foot is protruded the margins of the mantle are much enlarged to form muscular, thin-edged scrapers or valves, Figs. 1 and 2, *c*, that keep mud from being drawn or forced into the shell when the foot is withdrawn and the shell is forced down into the mud. It will be convenient to refer to this portion as the collar. The collar is very sensitive to touch and when stimulated is drawn tightly against the sides of the foot. When the foot is withdrawn it turns in over the end and so closes the shell. Strong stimulation of the collar when in this position, causes the margins to be drawn still further in and thus reflected into the shell.

The siphons are the most exposed, and apparently the most sensitive to stimuli of any portion of the mantle. They are surrounded by sense tentacles and, in the expanded animal, protrude a short distance beyond the posterior end of the shell. Tentacles occur all over the branchial siphon and fringe its margin. The cloacal siphon has tentacles around it and on its sides but its edge is very thin and does not bear tentacles. When stimulated the siphons contract and are withdrawn between the posterior borders of the shell valves. As has already been mentioned, the stimulation of the siphons of a specimen that is embedded in the mud is the signal for its disappearance. A very slight touch, such as might be given by a drifting weed or a piece of dirt, will cause an instant withdrawal of the siphons but may not cause the animal to burrow. If the stimulation is repeated, burrowing is quite sure to follow promptly. When the animal is removed from the mud, stimulation of the siphons when not long continued simply cause their complete withdrawal and the closing of the shell with the foot retracted. Continued stimulation, especially when accompanied with or preceded by the stimulation of the tentacles around the ventral mantle opening, and with the animal held with the anterior end pointing down, cause the foot to be protruded, swelled at the end and withdrawn in a manner similar to the movements of burrowing. If the stimulations are continued, these movements are usually repeated until a dozen or more complete thrusts and withdrawals have been made.

The foot, which is also periodically exposed to external stimuli, is likewise very sensitive. Stimulating its surface causes its withdrawal but it is never thrown into burrowing activity as the result. When the foot is withdrawn, the collar closes in over it, and if stimulation has been more than slight the siphons are retracted and the shell is closed.

From the foregoing it will be seen that a reasonably strong stimulation of any portion of the exposed animal affects it as a whole and may cause either complete retraction into the shell and the contraction of the muscles that close the shell, or may institute movements that are intended for escape into the mud. The latter movements seem never to be caused by the stimulation of either

the foot or the collar, but only by stimulation, usually when in the proper position, of the posterior or ventral mantle region. The habits of the animal are such that these regions are most likely to give warning of the presence of enemies.

If instead of applying reasonably strong and repeated stimuli, such as would be caused by stroking or pricking, very light and short stimuli are given, such as may be given by barely touching a tentacle with the side of a needle, a different result may be obtained. With a specimen lying in a dish of sea-water it is possible, by repeated slight stimuli, to cause the siphons or the foot and collar to be withdrawn without visibly affecting other portions. The foot and collar are so intimately associated, touching each other as they do, that it is very hard to cause the retraction of one without the other, but it is possible to cause a marked change in one without appreciably changing the other.

Before considering reactions further it is desirable to give attention to the nervous system.

The three pairs of ganglia that are usually present in lamelli-branches are all well developed, but there are no other definite ganglia. There seem to be a few scattered ganglion cells about the branchial nerves and a few others in sensitive portions of the mantle, but on the whole the nerves, commissures and connectives are remarkably free from ganglion cells. Although small ganglia are reported to be present on the cerebro-visceral connectives of Solon,² a very closely related form, I find no trace of such ganglia in *Ensis*, either in the dissections of mature individuals or in the serial sections of individuals about two centimeters long.

The cerebral ganglia, Figs. 1 and 2, *cg*, lie directly ventral to the anterior foot muscles and anterior to the mouth. They are far apart and are connected by a narrow commissure, Fig. 2, *cc*, in which ganglion cells do not seem to be present. Each cerebral ganglion is joined to the corresponding visceral and pedal ganglion by connectives, Figs. 1 and 2, *cvc* and *cpc*, in neither of which are ganglion cells abundant. Posteriorly each cerebral ganglion sends a nerve to supply the labial palps of the same side, Fig. 2,

² Lankester's *A Treatise on Zoölogy*, part 5, Mollusca (Pelseneer); Cambridge Natural History, Mollusca.

lpn. Dorsally and anteriorly a nerve is continued to the corresponding anterior foot muscle, Fig. 2, *afn.* Anteriorly a large nerve that soon branches starts forward. A portion of the first branch of this nerve bends ventrally to the margin of the mantle lobe and is continued posteriorly as the circum-pallial nerve, Fig. 2, *cpn.* The remainder of this first branch is continued forward toward the collar. The second branch from this large anterior nerve, *aan*, supplies the anterior adductor muscle. It is not always given off at exactly the same point in different specimens, and it sometimes happens, as in the case of the specimen shown by Fig. 2, that the origins of the nerves on the two sides are not symmetrical. The examination of serial sections and physiological experiments both indicate that these are the only nerves that supply this large muscle. The remainder of the large anterior nerve is continued anteriorly and sends numerous branches to the collar region of the mantle. The nerves of the two sides are continuous in front of the anterior adductor muscle so a complete connection between the two cerebral ganglia is formed, just as the circum-pallial nerves connect the cerebral and visceral ganglia of their respective sides. It may be well to state here that, while such anatomical connections undoubtedly exist between these ganglia, repeated experiments have failed to show the possibility of sending a nervous impulse from one ganglion to another by either of these connections. Possibly neurones from the two ganglia overlap in their distribution so there may be more complete coördination between portions that work together.

Upon cutting the inner lamellæ of the inner gills where they are joined together, and pushing them to their respective sides, the visceral ganglia, Fig. 2, *vg*, are immediately seen. They lie just anterior to the posterior adductor muscle, sometimes, as in the specimen shown in Fig. 2, with their posterior ends overlapping the anterior border of the muscle. The visceral ganglia are closely fused, so there is only a slight constriction between them. The commissural fibers are distinctly visible in sections but ganglion cells cover them entirely. As already indicated each visceral ganglion is joined to the corresponding cerebral ganglion by a connective, Figs. 1 and 2, *cvc*, that runs along the side of the body,

and by a circum-pallial nerve, *cpn*, that follows the margin of the mantle lobe. There is no indication that sensory impulses ever travel from one ganglion to the other through the circum-pallial nerve. All of the cerebro-visceral association fibers seen to be contained in the cerebro-visceral connectives. Soon after leaving the visceral ganglion each pallial nerve gives rise to a branch that supplies the posterior adductor muscle, Fig. 1, *pan*. It is then continued posteriorly and ventrally, sends many branches to the siphonal region and then turns anteriorly along the border of the mantle as the circum-pallial nerve, *cpn*, which joins the cerebral ganglion. What service is performed by this connection is not clear unless it is to afford overlap for the distribution of the motor fibers from the two ganglia to the margin of the mantle. Sensory fibers from the siphons all seem to go to the visceral ganglia, and from the tentacles around the ventral opening in the mantle, to the cerebral ganglia. For the siphons this is easily determined by cutting the pallial nerves between the nerves that supply the siphons and the visceral ganglia, when stimulation of the siphons causes no response, and for the tentacles around the ventral opening by cutting the circum-pallial nerves between the tentacles and the cerebral ganglia, after which stimulation of the tentacles causes no response. If the cut is made between the siphons and the ventral tentacles, the effect of stimulating either portion seems entirely normal. The motor fibers of these nerves are hard to experiment with but it is evident that most of the mantle muscles posterior to the ventral opening are supplied by fibers from the visceral ganglia. Anterior to this opening, along the path of the circum-pallial nerves, the muscles are not very well developed. A branchial nerve, Fig. 2, *bn*, leaves each visceral ganglion to pass anteriorly and laterally to the united lamellæ of the corresponding pair of gills. The physiology of these nerves has not been studied. Upon stimulation of the isolated visceral ganglia, slight contractions of the posterior foot muscles have been observed, but this may have been caused by escaped current from the electrodes. Nerves from the visceral ganglia to these muscles have not been found. The supply of nerves to the heart and the cardioinhibitory action have not been studied.

The pedal ganglia lie in the foot, very near the point where its dorsal border joins the visceral mass, Figs. 1 and 2, *pg.* They are more deeply embedded than are the other ganglia, but parts of them may sometimes be seen when the foot is pressed ventrally and posteriorly. To expose them it is necessary to remove the overlying tissue, which includes a thin layer of muscle. Like the visceral, the pedal ganglia are closely fused and their connecting commissure is covered by ganglion cells. The cerebro-pedal connectives, Fig. 2, *cpc*, may be seen throughout their extent without cutting. Like the other connectives they seem to be free from ganglion cells. The pedal ganglia supply the nerves to the foot. From each ganglion three large nerves pass toward the end of the foot and one or two extend ventrally. From the postero-ventral surface of each ganglion a nerve passes posteriorly and laterally to the side of the foot. The pedal nerves are not easily reached without rather extensive dissection and their individual actions have not been studied. As they are the only nerves to the foot they must contain both motor and sensory fibers.

In stimulating the different ganglia directly, it was found that an electric stimulation that could just be distinctly felt by the tongue was most satisfactory as it did not cause mutilation and the results did not give evidence of escaped current.

With all commissures and connectives intact, the stimulation of any ganglion visibly affected the whole animal, but the relative time of the contraction of different parts differed according to the ganglion stimulated. Thus when the visceral ganglia were stimulated, the siphons responded immediately, the collar and anterior adductor muscle later, and the foot slightly later still. This could be noticed without the use of recording instruments and indicates that an appreciable time is taken in transferring from one set of fibers to another; much longer than in the higher animals. Organs connected directly with the ganglia stimulated always responded first and those that were stimulated through association centers later.

The majority of the experiments performed were to determine:

- (1) The organs that received nerves from each pair of ganglia.
- (2) Whether each pair of ganglia individually govern the move-

ments of the organs it supplies with nerves, or whether some of the ganglion are accessory and dependent. (3) Whether all connectives carry impulses in both directions. (4) How far it was possible to have impulses transferred through association centers that would not normally be concerned with the impulses in uninjured animals.

Stimulation of the ganglia directly and of the nerves that leave the ganglia, and stimulation of sensory areas and the nerves that supply the sensory areas were all used.

In discussing the various nerves that leave the ganglia, mention has been made of the organs they supply, so it is only necessary to summarize here. When the cerebral ganglia are separated from the other ganglia by cutting the cerebro-pedal and cerebro-visceral connectives and the circum-pallial nerves,³ stimulation of the ganglia causes contraction of the anterior adductor muscle, the anterior foot muscles, the collar and at least a portion of the margin of the mantle. The functions of the nerves to the labial palps were not determined.

When the visceral ganglia are separated from the others by cutting the cerebro-visceral connectives and the circum-pallial nerves, stimulation of the ganglia causes contraction of the siphons and of at least a portion of the mantle margins, and feeble contractions of the posterior adductor muscle. Slight contractions of the posterior foot muscles have also been observed, but as the ganglia lie very near them and no nerves have been found entering them from these ganglia, it seems probable that the slight observed contractions were due to escaped current. The posterior adductor muscle when stimulated directly did not contract more than when the ganglia were stimulated. It seems that it does not function much in closing the shell. Its office is satisfactorily filled by the thickened, united, posterior margins of the mantle lobes.

When the pedal ganglia are separated from the others by cut-

³ Although the circum-pallial nerves did not seem to be able to carry impulses from one ganglion to the other, they were cut in these experiments to make sure that the ganglia were isolated. They were cut far from the ganglia that were to be experimented upon, so the effect of their motor fibers could be determined.

ting the cerebro-pedal connectives, stimulation of the ganglia causes vigorous contractions of the whole foot, including the anterior and posterior foot muscles. It was not determined whether the foot muscles were affected throughout or only in part. They become so intimately connected with the general musculature of the foot that a complete contraction of the foot would necessarily involve them.

The above experiments show the organs that are supplied with motor nerves from each pair of ganglia, but they do not indicate whether the contraction was in each case caused by stimulating cells in the ganglia themselves or by stimulating fibers that might be passing through them from other ganglia.

By isolating the different ganglia and stimulating sensory areas connected with them, motor cells can be proved to be present in the cerebral and visceral ganglia. Stimulating the sensory surfaces was generally accomplished by stroking with the point of a seeker or pencil. If muscular organs connected with the ganglia contracted upon such stimulation, motor cells must be present in the ganglia.

After cutting the cerebro-visceral and cerebro-pedal connectives and the circum-pallial nerves (the latter near the siphons), stroking the tentacles around the ventral mantle opening caused contraction of the anterior adductor muscle, and both sides of the collar. After separating the visceral ganglia from the others by cutting the cerebro-visceral connectives and the circum-pallial nerves (the latter near the cerebral ganglia), stroking the siphons apparently caused slight contractions of the posterior adductor muscle and strong contractions of both sides of the posterior margins of the mantle. Stimulation of one of the pallial nerves electrically, caused the siphons, posterior adductor and both of the posterior mantle margins to contract. These experiments indicate that sensory cells end in both the cerebral and the visceral ganglia and that stimulating these fibers causes disturbances in the motor cells in the same ganglia that cause the contractions mentioned.

With the pedal ganglia results are not so easily obtained. When these ganglia are separated from the others by cutting the cerebro-pedal connectives, the foot immediately loses its rigidity, and any

amount of stimulation of the surface of the foot, electrically, chemically or mechanically results only in the contraction of muscle fibers in the immediate vicinity of the point of stimulation. The foot never makes a movement as a whole and will remain motionless for hours, probably until it dies. This seems to mean either that there are no motor cells in the ganglia or that the sensory fibers have no endings or collaterals in the pedal ganglia but are continued directly through these ganglia to the cerebral ganglia. I am inclined to believe that motor cells are present in the pedal ganglia and that the sensory fibers pass directly through them without endings or collaterals, for the following reasons: (1) Microscopically the ganglia show an abundance of ganglion cells and it seems more reasonable to believe that, in such a muscular organ, they are not all sensory, especially as the action of sensory cells so placed, if motor are not present, would have to be referred to the cerebral ganglia before movement could be effected. (2) When the cerebro-pedal connectives are cut the foot responds with contractions. These have the character of tetanic contractions that would more probably come from the action of disturbed nerve cells than from the single stimulus caused by cutting motor fibers. If such movements could be caused by the stimulation due to cutting fibers only, then the cutting of the pedal nerves (below the pedal ganglia) should cause them, but beside the single twitch caused at the instant of cutting no movements follow this operation. (3) If one of the cut cerebro-pedal connectives is stimulated, the foot as a whole, both sides, responds, apparently with a complete, normal contraction. The course of the fibers in the ganglia have not been traced, but the effect is not what we would expect if the action is the result of the stimulation of only half of the motor fibers that go to the foot. It is much more easily explained by supposing that impulses have been sent to association cells which cause the motor cells of the foot, contained in the pedal ganglia, to act. Stimulation of the nerves that leave one of the pedal ganglia, after the pedal ganglia have been removed, does not cause complete contraction of the whole foot, as it should if the ganglia themselves have had no effect.

Whatever the arrangement, there can be no question that the

pedal ganglia are deficient in originating power, and that when the pedal are separated from the cerebral ganglia the foot will not by itself, or as the result of surface stimulation, execute movements more than are to be accounted for by the local direct stimulation of muscle fibers. To make sure that the movements did not come from stimulating the ganglia, they were entirely removed and still stimulation of the surface of the foot gave exactly similar contractions.

From the foregoing experiments it would seem that both cerebral and visceral ganglia are able to receive impulses and to direct the movements of certain organs with which they are connected, when they are entirely separated from the other ganglia, and that the pedal cannot act by themselves. This is somewhat surprising but possibly the habits of the animal may account for it. Apparently the cerebral ganglia are central for the nervous system. This is indicated by their connections with the other ganglia as well as by experiments. They would then have charge of the special activities of the whole animal, as well as of the special organs in their immediate vicinity which they supply with nerves. The visceral ganglia govern over organs that are in constant activity, organs that must give warning of the approach of enemies. They must give warning to the cerebral ganglia and then be ready to cover the retreat by closing and withdrawing the siphons and contracting the posterior margins of the mantle and posterior adductor muscle. The cerebral ganglia may now take charge of the advance with the aid of the efficient accessory pedal ganglia. They have little more to do during periods of burrowing. During the life of the animal the foot is not in a position that it will be called upon to give such an alarm very often if ever, and during burrowing the cerebral ganglia can devote nearly their whole attention to the process. It is desirable in directing a retreat of this kind to have a general in charge that is in constant communication with outposts that may give information regarding the enemy. The cerebral ganglia have such communications; the pedal ganglia only indirectly.

The cerebral ganglia are the only pair that are far enough apart to allow the cutting of the connecting commissure without injuring

the ganglia themselves. With these ganglia the operation is very simple as they are connected by a long narrow commissure that is distinctly visible throughout its length. With the commissure cut it was found that certain activities were delayed or otherwise interfered with. Stimulating either ganglion apparently caused complete contraction of the foot. The same result was also obtained when the cerebral ganglion that was not to be stimulated was removed previous to the experiment, or, as already noted, if both cerebro-pedal connectives were cut and the cut end of one of them was stimulated. These experiments again show that to cause the action of the foot it is only necessary to stimulate one pedal ganglion, which sets up the necessary association impulses. If both cerebral ganglia had been left connected with the pedal or visceral ganglia, although separated from each other, it would have been possible that stimulation of one resulted in the stimulation of the other through the pedal or visceral ganglia. Similar experiments were tried to determine the effect upon the visceral ganglia when one cerebral was stimulated after the cerebral commissure had been cut. The siphons and posterior mantle margins of both sides always contracted completely, even though the unstimulated cerebral ganglion was separated by cutting connectives, or was removed. The results were thus similar to those obtained for the pedal ganglia.

With the cerebral commissure cut and the two sides of the collar separated to guard against a possible transfer of impulses through the anterior pallial nerves, although no evidence could be found that such transfer of impulses could be made when the nerves were intact, moderate stimulation of one side of the collar caused only the contraction of the same side of the collar with imperfect contractions of the anterior adductor muscle and possibly slight contractions of the anterior foot muscle of the same side, with the usual retraction of the foot siphons, etc. Strong and continued stimulation however caused contraction of the other side of the collar as well. Evidently the impulses that affected the cerebral ganglion that has control of this side of the collar, must have passed by way of either the pedal or the visceral ganglia. Experiment indicated that the impulses can be transmitted either way but

that the response is quicker and more marked by way of the pedal than by way of the visceral. Much the same results were obtained by stimulating the tentacles on one side of the ventral mantle opening after the cerebral commissure was cut, as were obtained by stimulating one side of the collar.

Other experiments were tried to determine to what extent impulses can be made to travel over association fibers in other than what would seem to be the usual ways. It was found that stimulating one ganglion of a pair readily affected to its fellow ganglion and that the disturbance could be readily passed on from this ganglion to others provided the transfer was of a nature that was probably usual. For example, if the right cerebro-visceral connective was cut and the right posterior pallial nerve was stimulated, all of the organs connected directly with the visceral ganglia on both sides responded, and, a little later, the organs connected with both sides of the cerebral ganglia and the foot responded. If the left cerebro-pedal connective of the same specimen is also cut and the same stimulation is given, the response of the foot is delayed slightly but not long. In the last case it has been necessary to send impulses from the right to the left visceral ganglion, from the left visceral ganglion to the left cerebral ganglion, from the left cerebral ganglion to the right cerebral ganglion, and from this to the right pedal, which in turn must stir the left pedal to action with it. It will be noticed that all transfers in this experiment are in directions that may be supposed to be usual, either from one ganglion to another of a pair, or by way of warning from the visceral to the cerebral, or from the cerebral to the foot. These impulses are sent so readily that in one case it was found that by stroking the siphons of a specimen that had been operated on in the manner described, regular burrowing movements were instituted. Stimulation of the tentacles on the left side of the ventral mantle opening gave results almost as quickly as on uninjured specimens. Here again the impulses from one ganglion to another are in usual directions.

On other specimens, when the left cerebro-pedal connective was cut and the left side of the collar was stimulated, the foot responded without delay. Impulses were moving in usual direc-

tions. If now the cerebral commissure is also cut and the left side of the collar is then stimulated, or for that matter if the left cerebral ganglion is stimulated directly, the foot responds with convulsive contractions only after considerable delay. In some cases no response could be obtained. In this experiment impulses must be passed from the left cerebral around through the visceral to the other cerebral and from this to the pedal before the foot was stimulated. The path cannot be considered usual and the action is both delayed and modified. It is interesting to find that the centers are able to respond at all in this roundabout and unusual way.

SUMMARY

1 This form is very satisfactory for experimental study of the physiology of the nervous system because of its shape and activity, and the ease with which its nervous system may be seen and operated upon.

2 Continued stimulation of any portion of the body will in time have its effect on all of the ganglia.

3 Certain organs like the siphons, collar and foot, may be so gently stimulated as to cause them to be withdrawn without disturbing organs that receive their nerves from other ganglia.

4 The relation of ganglia of a pair is quite intimate. Stimulating nerves connected with one causes organs connected with both to respond promptly.

5 Association fibers by which ganglia communicate with each other are found only in commissures and connectives. Although the anterior pallial nerves are united so that a connection is formed between the cerebral ganglia, and the circum-pallial nerves connect the cerebral and visceral ganglia of corresponding sides, there is no evidence that the ganglia are able to communicate through them.

6 Both cerebral and visceral ganglia are provided with sensory and motor cells. The pedal ganglia are apparently dependent upon the cerebral for initiative. When the pedal ganglia are isolated from the others, stimulation of the surface of the foot causes only local responses due to the direct stimulation of muscle fibers. It would seem that the sensory neurones have neither end-

ings nor collaterals in the pedal ganglia but are continued to the cerebral ganglia.

7 Impulses may pass in both directions through any of the commissures and connectives.

8 Stimulation may cause impulses to be sent by roundabout connections when the usual connections are destroyed, but the stimulation must be of considerable duration and the result is often considerably delayed.

University of Maine

January 7, 1908

EXPLANATION OF PLATE

The anatomy of Ensis directus, Con.

Fig. 1 A specimen as seen from the right side with both valves of the shell, the right lobe of the mantle, the right labial palps and the right gills removed. Represented with the siphons and collar extended and the foot slightly protruded. A very common position. Drawn from the study of dissections and serial sections and enlarged to about one and one-third natural size.

Fig. 2 A specimen as seen from the ventral surface with the mantle margins cut and the shell valves wide apart, and the foot forced posteriorly and to the right side of the animal. The inner lamellæ of the inner gills have been separated and pushed to the sides. The ganglia and nerves in this figure have unintentionally been made a little too large. Drawn from dissections with a few details added from the study of serial sections. Enlarged to about one and one-half natural size.

<i>aa</i>	anterior adductor muscle	<i>h</i>	heart
<i>aan</i>	anterior adductor muscle nerve	<i>lp</i>	labial palp
<i>af</i>	anterior foot muscle	<i>lpn</i>	labial palp nerve
<i>afn</i>	anterior foot muscle nerve	<i>m</i>	mouth
<i>apn</i>	anterior pallial nerve	<i>pa</i>	posterior adductor muscle
<i>bn</i>	branchial nerve	<i>pan</i>	posterior adductor muscle nerve
<i>bs</i>	branchial siphon	<i>pfm</i>	posterior foot muscle
<i>c</i>	collar	<i>pg</i>	pedal ganglion
<i>cc</i>	cerebral commissure	<i>pn</i>	pedal nerves
<i>cg</i>	cerebral ganglion	<i>ppn</i>	posterior pallial nerve
<i>cpc</i>	cerebro-pedal connective	<i>r</i>	rectum
<i>cpn</i>	circum-pallial nerve	<i>s</i>	crystalline style
<i>cs</i>	cloacal siphon	<i>st</i>	stomach
<i>cvc</i>	cerebro-visceral connective	<i>vg</i>	visceral ganglion
<i>f</i>	foot	<i>vm</i>	visceral mass
<i>g</i>	gill	<i>vo</i>	ventral mantle opening

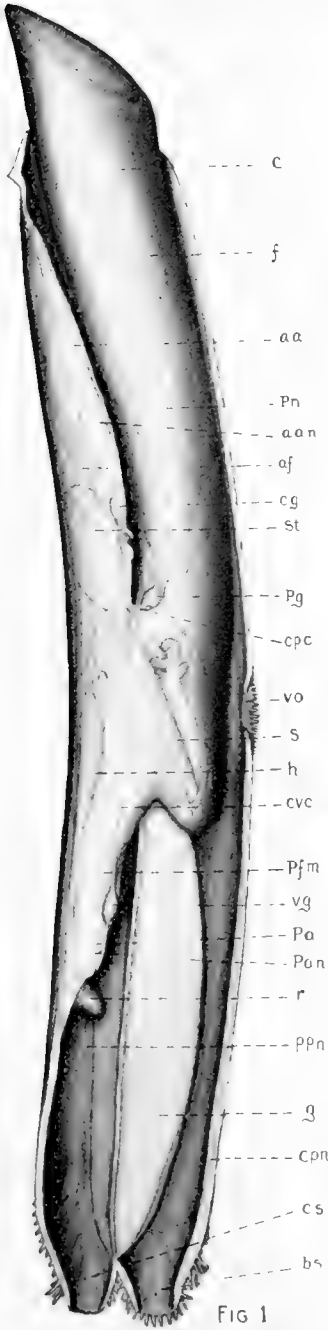


FIG 1

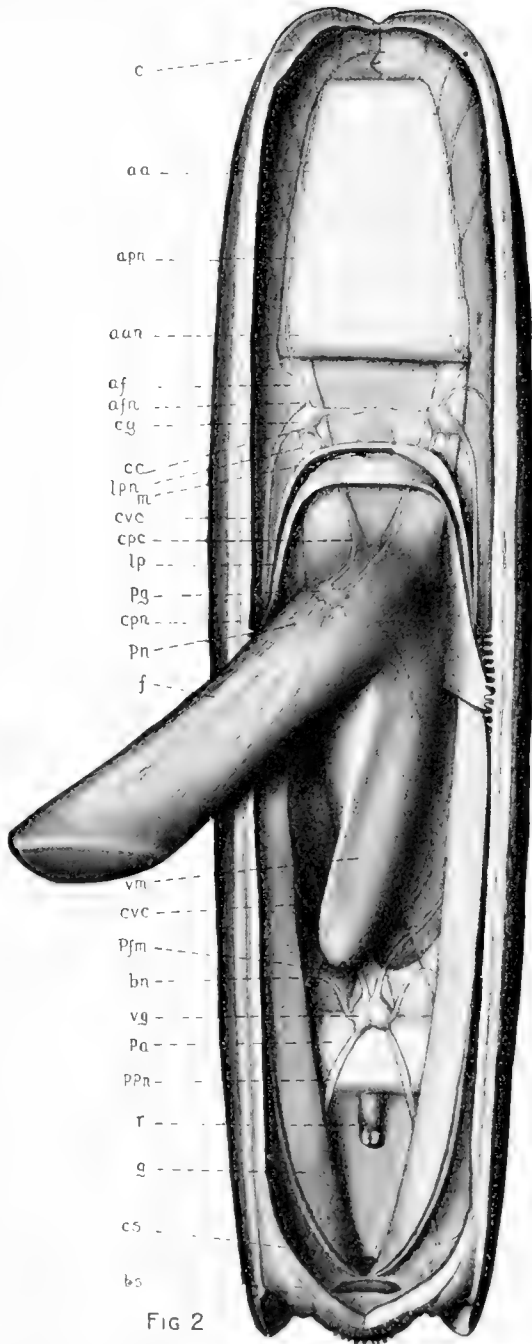


FIG 2

THE INFLUENCE OF GRAFTING ON THE POLARITY OF TUBULARIA

BY

FLORENCE PEEBLES

WITH TWENTY-SIX FIGURES

The experiments made by Loeb, Driesch, Morgan, and others, have demonstrated that by closing the oral end of a piece of the stem of *Tubularia* the development of the aboral hydranth is hastened. The same result was obtained by Morgan ('03) when he bent long pieces in the middle, or ligatured them so that the cœnosarc of the two ends was completely separated. Morgan and Stevens ('04) have shown further, that the formation of a hydranth at the aboral end of a piece produces a change in that region, so that when this hydranth is removed, the piece is more likely than before to develop another aboral hydranth.

The object of the experiments described in this paper was primarily to determine what influence grafting exerts upon the polarity of *Tubularia mesembryanthemum*, but one experiment led to another until the investigations extended to a study of some of the factors of regulation.

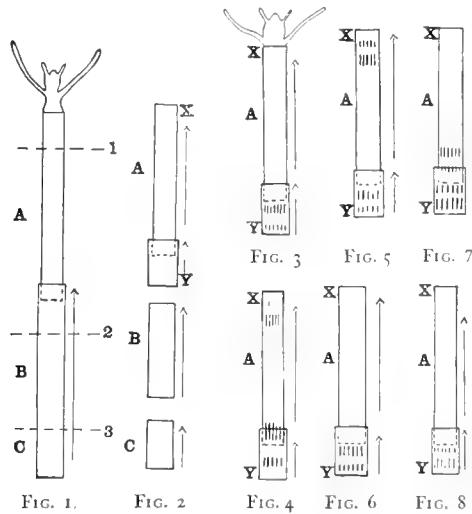
Last spring it was my privilege, through the generosity of the "Association for Maintaining the American Woman's Table at Naples," to spend two months at the Zoölogical Station, during which time I carried on the experiments described in the following pages. It gives me great pleasure to express here my gratitude to the Association, and also to Prof. Anton Dohrn, and the other members of the staff at Naples, for the courtesies extended to me during my stay.

In earlier experiments in grafting ('00 and '02) two components having the same diameter were selected, the two cut surfaces were applied, and held together until the cœnosarc united. This method proved so tedious that a new one was adopted. In

order to use this method one component must be slightly smaller than the other so that one end of the smaller one may be inserted in an end of the larger one. The two components were usually telescoped so that they lapped about one millimeter.

I TWO LONG PIECES GRAFTED TOGETHER IN THE SAME DIRECTION

Experiment I. The first series of experiments consisted in grafting together two pieces from the same region of two different individuals, so that the aboral end of one piece was inserted in the oral end of another piece from which the hydranth had just been



removed. Each piece measured about 3 cm. (Fig. 1), not including the hydranth of the distal piece which was not removed until the day after the graft was made. The first cut (1) removed the old hydranth and about 4 mm. of the stem from the distal component, the second cut (2) was made through the proximal component a short distance back of the line of union, and the last cut (3) removed the basal end from the proximal component. These three pieces (Figs. 1 and 2) I shall call respectively *A*, *B* and *C*. Their individual behavior after this second operation will first be considered, and then they will be compared in order to see the relative rate of development of the hydranths.

(*A*) The piece designated *A* (Fig. 2) consisted of the major part of the distal component, with a short distal piece of the proximal component grafted in the same direction, on its aboral end. If the long and short piece (Fig. 2, *A*) act as one, we should expect a new hydranth to form first at the oral end (Fig. 2, *X*) and later at the aboral end (Fig. 2, *Y*). If, however, the two components retain their individuality this result would not follow, for the oral end of the small component and the aboral end of the large component have had a start of twenty-four hours.

Forty-seven grafts were made, the results from these are given below in the table.

TABLE I A

Hydranths at <i>X</i> first, later at <i>Y</i>	18
Hydranths at <i>X</i> none, later at <i>Y</i>	14
Hydranths simultaneously at <i>X</i> and <i>Y</i>	6
Hydranths first at <i>Y</i> , later at <i>X</i>	6
Hydranths at <i>Y</i> , none at <i>X</i>	3
<hr/>	
Total number of grafts.....	47

From this table it is evident that the oral end of the long piece is the region that produces the greatest number of hydranths, and that when they form at both *X* and *Y* (Fig. 2) they usually develop at the oral end of the long piece before they appear at the aboral end of the small piece (Fig. 3). About one-half of the hydranths forming at *Y* came entirely from the small piece, but in the reverse direction therefore they are aboral hydranths (Fig. 3). In the remaining one-half both components took part, the proximal row of tentacles appearing in the long piece, and the distal row in the short piece (Fig. 4). A large number of the grafts (almost one-third) developed neither hydranths nor stolons at *Y*. Six developed new hydranths simultaneously at *X* and *Y*. Of these, four of the aboral hydranths were composed partly of one, partly of the other component (Fig. 4), and two developed in the small piece only (Fig. 5). Twenty per cent of the grafts continued development at the line of union showing however the influence of the second operation, for the hydranths instead of taking their usual direction emerged from the cut end (Fig. 6). In those that

formed double hydranths, one at the aboral end of the long piece, and one on the short piece, the original direction of the small component was always maintained (Fig. 7). In one graft where the short piece developed a hydranth in the original direction (Fig. 8) it finally pushed the graft apart and emerged.

(B) Loeb ('04) has described a series of experiments on *Tubularia* in which he claims that the polarity was reversed. His experiment was as follows: A long piece cut from the stem was ligatured near the oral end. New hydranths developed at both ends. After the hydranths had emerged from the perisarc he cut a piece out of the region aboral to the ligature. Two days after the second operation ten pieces had formed aboral hydranths and only five had oral hydranths. From this result he concluded that the polarity was reversed. In other words the aboral end having formed a hydranth once, after the second operation, formed one again because the hydranth-forming material had been carried to that end.

In my experiment the piece *B* (Figs. 1 and 2) corresponds in position to the piece described by Loeb. Instead of waiting until a hydranth had formed at each end, the piece from which it was cut was united with another piece at its oral end and given a start of twenty-four hours at its aboral end. Some changes must have been going on at these two ends as some of the pieces did not behave like corresponding pieces cut immediately from stems that had not been grafted. The results are given in the following table:

TABLE 2 B

Oral hydranths not followed by aboral.....	23
Oral hydranths followed by aboral.....	15
Oral and aboral hydranths simultaneously.....	7
Lost.....	2
	—
Total number of pieces.....	47

The majority of pieces behaved like similar pieces in the control experiment, but in a few cases (seven out of forty-five) the development of the aboral hydranth was hastened. Not one piece developed a hydranth *first* on the aboral end.

(C) At the time of removal of the basal piece (Figs. 1 and 2) no sign of the tentacles' ridges was visible, and it was possible in only a few cases to see circulation at the aboral end. It does not follow however from this that there was no preparation for a hydranth. It was difficult to keep these short pieces oriented, therefore the results are meager. I have thrown out all those about which there was any doubt, so that the table gives a record of only twenty pieces.

TABLE 3 C

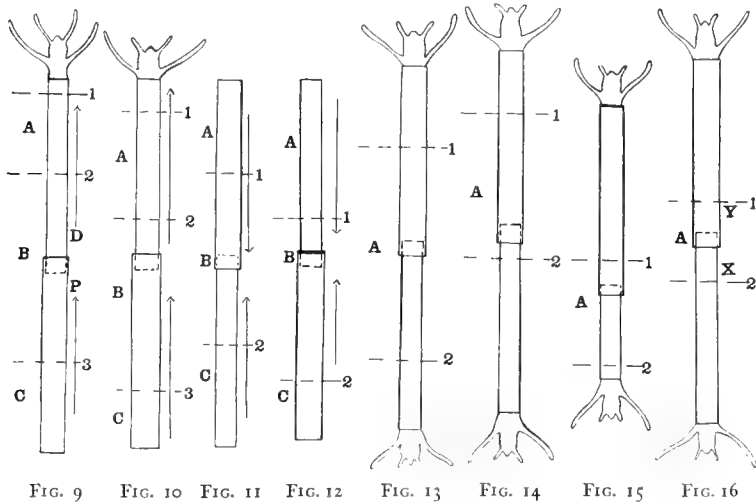
Hydranths at the aboral end first.	8
Hydranths at the oral end first.	6
Hydranths at the aboral and oral ends simultaneously.	2
No hydranths.	4
<hr/>	
Total number of pieces.	20

On the eight pieces forming aboral hydranths first, the oral hydranths followed very quickly, a much shorter space of time intervening than between oral and aboral hydranths when the oral form first. These double ended pieces were kept until the hydranths dropped off, and a second set developed. In spite of the fact that those on the aboral end developed first, the second set appeared on the oral ends first, the aboral forming two days later. The pieces thus returned to their original polarity.

It is hardly possible to draw conclusions from such a small number of pieces, but the results are sufficient to show that the aboral hydranths after they are once developed do not exert enough influence over the region from which they come to establish permanently a marked polarity.

In order that we may compare one series as a whole, I have made the following table giving the relative time of formation of the hydranths on the pieces *A*, *B* and *C*. The region of the graft is indicated in *A* by the short horizontal line. The numbers placed beside the pieces show the order of emergence of the hydranths, the letter *S* stands for a stolon. Where no letter or number appear there was no regeneration. The arrows point toward the oral end of each piece.

third cut divided the proximal component in half. The piece *A*, between the first and second cuts, differed from *C* not only in position, but in time of exposure of the ends. Both ends of *A* were exposed at the same time, but the aboral end of *C* was exposed twenty-four hours before the oral end. In this experiment the graft is in the middle piece (Fig. 9, *B*).



Taking the piece *B* first as it is made of the two equal components grafted together in the same direction (Fig. 9) and comparing its later behavior with that of *A* in the first experiment, we find that the effect of the second operation is not so evident. Twenty-four hours after the piece had been separated from *A* and *C*, on 75 per cent of the pieces new hydranths were developing on the oral ends of the basal component, and on less than 50 per cent of the grafts oral hydranths were appearing on the distal components. The two components rarely acted as one piece, for one or more hydranths usually developed in the region of the graft. In the following table the results from twenty grafts are given. That part of the graft which came from the distal component is designated as *D*, that from the proximal component as *P* (Fig. 9).

If we compare Table 5 with Table 1, it will be seen at once that there is no marked difference in the number of hydranths formed

on each side of the line of union. A difference was observed in the number of aboral hydranths on the distal piece, although this is not shown in this table. Eight out of the twenty formed aboral hydranths in this experiment where the two components were of the same length, and in the first experiment where the proximal component was much shorter than the distal, only five out of twenty-seven, formed an aboral hydranth on the longer piece.

TABLE 5

Hydranths on the oral end of <i>D</i>	9
Hydranths on the oral end of <i>P</i>	11
Hydranths on aboral end of <i>D</i>	8
Hydranths on aboral end of <i>P</i>	4
<hr/>	
Total number of pieces.....	20

Before combining the results from the three pieces the behavior of pieces *A* and *C* (Fig. 9) will be considered very briefly. These two pieces were about the same length, but they were not taken from the same region of the original stems, and while the oral and aboral ends of *A* were exposed at the same time, those of *C* were not. The aboral end of *C* had a start of twenty-four hours. In Table 6 the results from twenty pieces are given.

TABLE 6

Oral hydranths	A 16	C 3
Aboral hydranths.....	A 2	C 15
Aboral stolons.....	A 1	C 0
No regeneration.....	A 1	C 1
<hr/>		
Total number of pieces.....		20

In order to compare the behavior of the three pieces Table 7 was made showing the results from twenty-one of the double components. In these grafts the second operation followed the first after a period of twenty-four hours.

Experiment 3. In a third series of experiments, instead of allowing the grafts to remain twenty-four hours before the second operation, the time was shortened, and after three to four hours, the pieces were isolated. The results are given in Table 8.

These results show a great difference in the region of the line of union. The number of hydranths formed there was greatly

TABLE 7

[illegible]

reduced, the double pieces resembling a single one in their behavior. Pieces of the same length as *A* and *C* were cut from corresponding regions of stems which had not been grafted. A com-

TABLE 8

[illegible]

parison of their behavior with that of these pieces cut from the grafted stems shows a marked difference in the number of hydranths

developed. A much larger per cent of aboral hydranths formed on *A* in the control, and also more oral hydranths on *C* in the control. This seems to indicate that the process of hydranth formation at the grafted ends of these pieces affects their later behavior.

Table 7 and Table 8 show very definitely, that the results after the second operation are not modified by those following the first unless the period between the two operations is sufficiently long for them to get a start. In the first place (Table 7) the number of oral hydranths on the distal half of the original distal component, is much larger than that on the proximal half, while there are more aboral hydranths on the proximal half than there are on the distal half. This is not the case (Table 8) when the second operation follows the first after a very brief period. Secondly, the number of aboral hydranths on the proximal end of the original proximal component is much larger than the number on the distal half of the same component, and the number of oral hydranths on its distal half is greater than on the proximal half. This is not the case when the time between the first and second operations is reduced.

Experiment 4. In the fourth series of experiments the two components were grafted together in the way described above, but this time the level of the second cut was changed, making the piece *A* (Fig. 10) consist of the greater part of the distal component while *B* (Fig. 10) was made up of a short basal piece of the distal component grafted on the oral end of the proximal component. The third cut (Fig. 10, 3) was made nearer the aboral end of the proximal component thus making *C* shorter than in the preceding experiment. A series of sixteen grafts of this description are represented in Table 9.

If we compare the rate of development of hydranths on *A*, *B*, and *C* we find oral hydranths on *A* and *C* appearing at about the same time, also those on the oral and aboral ends of *B*. The per cent of hydranths on the oral and aboral ends of *A* and *C* was about the same as that in Experiments 1 and 3. The proportion of double hydranths at the region of the graft was larger, and also the number of aboral hydranths on the distal end of the proximal component. When this experiment is modified by decreasing the time

between the first and second operations (Table 10) no hydranths formed on the oral end of the proximal component in piece *B*, but a large number of the distal short pieces formed hydranths. *A*

TABLE 9

2	2	1	2	1	2	2	2	2	2	2	2	2	1	2		A
			3	2	2			2					3	3		
1	2			1	2	2							2	1	2	
	1	2	3		2	2	1	2	1	3	3	2	1		1	B
	2	1	4	2		2	2			4	4	2				
		3	3			2	2	2	2			2	2	2	2	
1	1	1	1		1	1	1	1		1	1	1	1	1	1	C

very small number of hydranths developed on the aboral ends of *A*, in this series not one. The oral hydranths on *A* and *C* appeared about the same time.

TABLE 10

1	1	1	1	1	1	1	1	1	1	1		A
		1	S	1	1	1		1				
												B
2	2	2	1	2		2	1				3	
1	1		1	1		1	1				1	
2							2	1			1	C

2 TWO LONG PIECES GRAFTED TOGETHER BY THEIR ORAL ENDS

Experiment 1. Two pieces of stem, each measuring about 3 cm., were grafted together by their oral ends (Fig. 11). The grafts were left undisturbed for twenty-four hours, then the double piece was cut at two levels (Fig. 11, 1 and 2) so that each component was halved. The distal halves of each were united by their oral ends forming the piece *B* while the proximal halves of each (*A* and *C*) had their oral ends exposed by a fresh cut, their aboral ends having a start of twenty-four hours. New oral hydranths formed on *A* and *C* at practically the same time, the aboral usually preceding the oral by a few hours, or forming simultaneously with it. The piece *B* developed a very small proportion of hydranths at its free aboral ends, but in nearly every graft double heads formed, one on the oral end of each component; these emerged and finally pulled apart. These oral hydranths were much slower in developing than the oral hydranths on the free ends of *A* and *C*. When the second operation followed a few hours after the first, the percentage of hydranths formed at the line of union was greatly reduced. The free aboral ends rarely developed hydranths at the same time, one usually preceded the other by six or eight hours. The oral hydranths on *A* and *C* in this experiment formed before the aboral hydranths with very few exceptions.

Experiment 2. In a second series of experiments in which the two components were grafted together by their oral ends (Fig. 12), the level of the first cut was changed so that *A* consisted of the major part of one component, while in *B* instead of the components being equal in length, one was much longer than the other (Fig. 12, *A* and *B*). A period of eighteen to twenty-four hours elapsed between the first and second operations. Table 11 gives the results from eighteen grafts. These practically represent the entire series of experiments so that it is unnecessary to give other tables.

If we consider the rate of appearance of the hydranths we see that the percentage of aboral hydranths is very large, and that they appear before the oral hydranths of the same piece with few exceptions. The number of hydranths on the oral end of the

large component of the graft is greatly reduced, while a relatively large number form on the aboral ends of the smaller component. The pieces *A* and *C*, upon which the aboral hydranths appeared before the oral, were kept until the first hydranths were lost, and new ones developed. Without exception, the second set of oral hydranths appeared first, even in cases where the aboral hydranths had developed one to two days earlier than the oral ones. This shows without doubt that the polarity is not permanently reversed.

Experiment 3. In this experiment the second operation followed the first after six hours. The results show that the aboral ends had not had a sufficient start to produce hydranths before

TABLE II

the oral ends. None of the grafts formed hydranths on their oral ends, instead, the majority developed a hydranth first at the aboral end of the short piece, and several hours later at the aboral end of the longer piece.

3 TWO LONG PIECES GRAFTED TOGETHER BY THEIR ABORAL
ENDS

It has been demonstrated by many observers that a piece cut from the stem of *Tubularia*, if sufficiently long, shows marked polarity, i. e., a hydranth develops first on the oral end of the stem and then later on the aboral end. I have shown that when two

long pieces are grafted together in the same direction, they may act as one piece, a hydranth forming first at *X* and later at *Y* (Fig. 2) but a large per cent form hydranths simultaneously at the oral ends of each component (Fig. 4). The question naturally arises as to the result if two pieces are grafted together by their aboral ends so that the two oral ends are exposed.

Experiment 1. Two pieces each measuring about 3 cm. with the hydranths still attached, were grafted together by their aboral ends (Fig. 13). At the end of twenty-four hours the hydranths were removed, together with 1 cm. of the stem. The graft (*A*) consisted of two components of equal lengths whose aboral ends had been united for twenty-four hours and whose oral ends were exposed at the same moment by a fresh cut. This experiment was repeated, with modifications, several hundred times. The results can not be combined in tables without much repetition, therefore I shall merely give one representative series.

TABLE 12

1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	2	2	1	1	1	2	1	↑
1											1	2	2	1	1	1	1	1	1	1	1	1	↓

In this series no hydranths formed at the graft line. In most cases the two components acted as one piece forming a hydranth at one end only, or first on one end, and then on the other. Whether stolons would have developed later, I can not say, for the pieces were kept only four or five days. At the end of that time although two sets of hydranths developed on some pieces there was no sign of stolon-formation or pushing apart at the aboral ends. That one piece is influenced by the other seems evident from the results. Only five grafts formed hydranths on each end at the same time, while ten produced one first at one oral end, and then after one to two days, at the other oral end. Ten more formed hydranths at one of the oral ends and nothing at the other.

Experiment 2. In a second experiment one component was cut close to the line of graft (Fig. 14, 2). In this way, it was thought,

current has nothing to do with the order of appearance of the hydranths on the ends of the grafts. We must seek an explanation elsewhere. I believe that it requires a large amount of energy to construct a new hydranth. In order to produce sufficient energy certain metabolic processes are set up. These processes must begin as soon as the wound closes. If the condition of the stem is such that sufficient energy can be produced, hydranths are formed at once, if not, the development is delayed until there is enough energy. When two pieces are grafted together, some of this energy is expended in healing the wound, and uniting the pieces. If there is a large enough quantity left over, or already in the pieces, hydranths develop at once at the two oral ends, but if there is not a large enough amount present, one end is delayed until the hydranth has been completed. This hypothesis may serve to explain the hastening of the aboral hydranth after one has been formed or is about to form in that region. If the hydranth has formed there may be energy left over, if it is about to develop there is a large amount of energy present. Under normal conditions some stems contain more energy, or vitality. The preceding tables show that from the two original components a large number of hydranths may develop as many as eight, while from others only one or sometimes none, appear. The conditions of the experiment are apparently the same; the results can be explained in no other way than that one individual possesses more energy than another. I do not believe that there is any one material whose presence modifies the result, it is the state of all the materials at the time of the operation.

3 GRAFTING A SHORT DISTAL PIECE ON THE BASAL END OF THE SAME STEM

In an earlier paper ('00) I described a series of experiments in which a short distal piece of the stem was grafted in a reverse direction, on the proximal or aboral end of a long piece. The results which I obtained from a small number of experiments, seemed to indicate that the long piece influenced the rate of development of a new hydranth on the short piece, for the tentacle ridges on the short piece did not appear until after the hydranths had

emerged from the oral end of the longer piece, a region which was nearer the basal end than the short piece. The number of experiments was almost too small to warrant any definite conclusion. I have repeated this experiment a number of times and have finally come to the conclusion that the major component does not influence the minor one unless it shares in the formation of the hydranth which develops at that end.

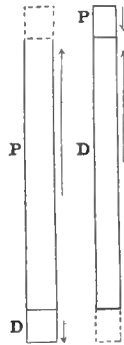


FIG 17 FIG. 18

Experiment 1. A short piece measuring about 1.5 mm. was cut from the distal end of a long (3 cm.) piece from a region near the hydranth. This small piece was then grafted on the aboral end of the long piece (Fig. 17) so that the oral end of each piece was exposed, the aboral ends united. Twenty-seven grafts are represented in Table 14. The dotted line shows the original position of the small piece.

TABLE 14

Diagram illustrating a sequence of 20 trials. Each trial is represented by a vertical line. The top of each line is labeled with a number (1 or 2), and the bottom is labeled with a number (1 or 2). A downward arrow is on the left. A 'P' is at the bottom right.

Trial	Top Label	Bottom Label
1	1	2
2	1	2
3	1	1
4	1	2
5	1	2
6	1	1
7	1	2
8	1	1
9	1	2
10	1	1
11	1	2
12	1	1
13	1	1
14	1	2
15	1	2
16	1	2
17	1	1
18	1	2
19	1	1
20	1	2

Eleven of the twenty-seven grafts formed a hydranth first at the oral end of the major component (*P*), and later at the oral end

of the minor component (*D*). Eleven developed oral hydranths on the long piece and nothing on the shorter one. Two developed hydranths on the short piece only, and two on the aboral end of the long piece only. In one case new hydranths appeared simultaneously on the two exposed ends. In all grafts where a long and a short piece are united, the formation of a new hydranth is always slower in the short piece. In six of the eleven grafts that developed the hydranth first at the oral end of the major component, the hydranth that formed later on the other end came partly from the long piece and partly from the short one, i. e., the distal tentacles developed in the minor component, and the proximal in the major component. In these the development was always slower.

4 GRAFTING A SHORT BASAL PIECE ON THE DISTAL END OF THE SAME STEM

In order to test the influence of a long distal piece on a short basal one, a second series of experiments was made. This time the hydranth was cut from a piece of stem measuring 3 to 4 cm. From the basal end of this piece a short piece (2 to 3 mm.) was cut off, and grafted in the opposite direction, on the oral end of the same stem (Fig. 18). The results from these experiments were not surprising. Here no influence seemed to be exerted by the major component, as the following table shows.

TABLE 15

↓	1	1	1	1	1	1	2	2	2	2	1	2	P
↑	1	1	1		1	1							D
				2	1		1	1	1	1		1	

No very definite conclusions can be drawn from this table, or from any of the other series in this experiment. The major component apparently took no part in the formation of the hydranth in the smaller piece. From constant observation of the behavior of grafts composed of a short and a long piece, I am inclined to believe that the size of the short piece has more to do with the rate

of regeneration than contact with the major component. The only cases where it seems to me we are justified in looking for the influence of one upon the other is where the hydranth is developed partly in the long piece and partly in the short one. This did not take place in any of the experiments.

5 THE INFLUENCE OF THE CURRENTS ON REGENERATION AFTER GRAFTING

Experiment 1. Two short pieces grafted in the same direction. It was suggested to me by Professor Morgan, that the current in the two pieces of which a graft is composed, may have something to do with the order of regeneration in the outer ends of grafts. Soon after a piece is cut from the stem of *Tubularia*, the wound closes, and rapid circulation begins. The current is easily seen coursing up one side of the piece and down the other. When two pieces are united the currents do not always flow from one piece into the other. Instead, the current may be seen flowing up one piece and turning at the line of union as if stopped by a membrane, and continuing down the other side. Frequently, however, the current continues to flow up one side and on into the other piece. In Fig. 19 (*E* and *F*) these two cases are shown. In *E* the current is continuous; in *F* it is separate in the two pieces.

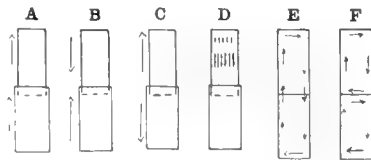


FIG. 19

Small pieces, measuring 1.5 to 2 mm. in length, were cut from different stems from a region at least 1 cm. back of the hydranth. One piece was inserted in the other so that the oral end of one overlapped the aboral end of the other (Fig. 19, *A*). At the end of twenty-four hours each graft was carefully examined under sufficient magnification to detect the direction of the currents. All of the grafts in which the circulation was continuous from one component to the other (Fig. 19, *E*) were put in lot *A*, while those in

which the circulation of one piece was distinctly separate from that of the other (Fig. 19, *F*) were isolated in lot *B*. Another lot (*C*) consisted of those in which the circulation was irregular, and the last (*D*) in which no circulation whatever could be detected. The rate of development of the hydranths is shown in Table 16.

TABLE 16

A		B			C				D		
↑	1	1	1	1	1	1	1	1	1	1	1
↑	2	2	2	2	2	2	2	2	2	2	2

The behavior of the grafts is practically the same whether the circulation is continuous or not. In each case a hydranth developed first on one piece and then on the other or on one piece only.

Experiment 2. Two short pieces grafted together by their oral ends. Pieces of the same length as those in the preceding experiment were grafted together by their oral ends, so that the aboral ends were exposed. They were separated as before into lots *A*, *B*, *C* and *D*. The results from one series are shown in Table 17.

TABLE 17

A					B		C				D		
↓	1	1	1	1	1	1	1	1	1	1	1	1	1
↑	8	1	1	1	2	1	1	1	1	1	2	2	1

Of these thirteen grafts four developed simultaneously on the aboral ends. Three formed one first on one end, then on the other, five formed one on one end and none on the other, and one developed a hydranth on one end and a stolon on the other. A control experiment was made in which single pieces, the same length as the entire graft, were cut. Out of eleven pieces, eight formed a hydranth on one end and nothing on the other; two developed a hydranth first on one end, and then on the other, and one developed a stolon on one end, and a hydranth on the other.

Experiment 3. Two short pieces grafted together by their aboral ends. These pieces were the same length, and taken from the same region of the stem, but were grafted by their aboral ends

(Fig. 19, *C*) so that the oral surfaces were exposed. A series of twenty-four grafts is represented in Table 18.

TABLE 18

A						B			C												D	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Out of twenty-four grafts, twelve developed a hydranth first on one end, then on the other in spite of the fact that in some the current was continuous in the two pieces and not in others. Only one developed hydranths simultaneously on the free ends. Eleven formed a hydranth on one end only.

These experiments, as a whole, show that double pieces usually form one hydranth only (Fig. 19, *D*), or first one and then another later on the other end, regardless of the direction of the graft, or the flow of the currents.

6 THE EFFECT OF INTERRUPTING THE NORMAL PROCESS OF HYDRANTH FORMATION

Driesch ('97) first showed, in his researches on *Tubularia*, that when the formation of the hydranth is interrupted by separating the two tentacle ridges shortly after they appear, the method of completing the hydranth is not always the same. He has described four methods of regulation: (1) The "Regenerationsmodus" where the hydranth emerges with the original proximal tentacles, and later develops a new distal row; (2) the "Ersatzanlagemodus" where the coenosarc in front of the proximal row elongates and a new distal row appears before the hydranth emerges; (3) the "Auftheilungsmodus" where the proximal tentacles divide, forming the distal row from their distal ends; (4) the "Auflösungsmodus" where the proximal row disappears entirely and a new anlage forms. I repeated these experiments ('00) suggesting that the difference in the method of completion of the hydranth on the proximal piece was due to the degree of differentiation of the primordia. If the distal row of tentacles was removed soon after the red material had begun to collect in the two rows, the fourth method was invariably followed, i. e., the first proximal row dis-

appeared and the complete new anlage developed. If on the other hand the two rows were separated later after they were well defined, the first method of completion was followed. The proximal piece is, therefore, as Driesch has shown, capable of completing itself in a distal direction. The small piece (*A*) bearing the distal row of tentacles, is also able to complete itself distally, but as far as I am aware no one has found that such a piece is capable of forming new proximal tentacles, thus completing itself in a proximal direction.

It seemed to me worth while to repeat this experiment in order to find out at what time the distal piece (Fig 20, *A*) becomes so highly differentiated that it is no longer able to complete itself proximally, and also to observe the other methods described by Driesch, especially the "Auftheilungsmodus" which I had never seen, although I had repeated the experiment more than a hundred times.

In order to find out the exact time when the small piece (Fig. 20, *A*) becomes too highly differentiated to complete itself, it was necessary to remove the tip of the stem before the tentacle ridges were visible. Driesch tried this on forty-five pieces, allowing about twenty-four hours to intervene between the first and second operations. Out of these forty-five pieces, thirty-seven developed one row of tentacles, two formed a double row, and six a complete hydranth without a stalk. Five of the six pieces that formed a complete hydranth were "zu gross," therefore he considers that there were only three out of forty which developed more than the distal row. He concludes that there is therefore a definite time before the anlage appears, when the character of the further development of the smaller tip is determined. In order to determine the exact position of the tentacle ridges before they are visible on the outside, it was necessary to measure a number of anlagen, then to take the average length. Fifty pieces were cut from different stems, and left undisturbed until the anlage was visible on each. Measurements were then made, first from the tip of the stem to the base of the proximal tentacles (Fig. 21, *P*), and second from the tip of the stem to the base of the distal tentacles (Fig. 21, *D*) the average length of *P* was 1.7 mm. of *D* .6 mm.

Experiment 1. Removal of the region of the distal tentacles before the ridges appear. The hydranth and about 5 mm. of the stem below it were removed from twenty-seven pieces of stems whose length averaged 3.5 cm. These pieces were left undisturbed for eighteen hours. At the end of this time the circulation in the oral end was rapid but no ridges had been laid down. A piece .7 mm. long was then removed from the oral end of each piece (Fig. 22, *A*), thus separating the region in which the distal tentacles would appear later, (*A*) from the longer proximal piece (*B*) on which the proximal row would have developed. The pieces were isolated and their later behavior observed. The following table gives the results from twenty-five pieces, two of the twenty-seven having been lost.

SERIES 1 A

Complete hydranth.....	5
Distal tentacles only.....	10
No regeneration.....	10
	—
Total number.....	25

By complete hydranth I mean distal and proximal tentacles, and reproductive organs. No stalk developed on these small pieces.

In the second series, twenty-two pieces were cut, the length of the distal piece (*A*) was increased from .7 mm. to 1 mm., thus including part of the region between the two rows of tentacles. The time between the first and second operations was the same as that in Series 1.

SERIES 2 A

Complete hydranth.....	9
Distal tentacles.....	9
No regeneration.....	4
	—
Total number.....	22

Comparing Series 1 and 2, it will be seen that with an increase in the length of the distal piece (*A*) there is an increase in the proportion of complete hydranths.

In a third series *A* measured .8 mm. This time the tip was removed as soon as the piece was cut from the stem, so that no time elapsed between the first and second operations.

SERIES 3 A

Complete hydranth	0
Distal tentacles	7
Double row	1
No regeneration	4
Total number	12

In this series one of the "incomplete" structures, i. e., a hydranth composed of two distal rows developed. This result is often obtained from short pieces. The piece *A* in this experiment was shorter than the short pieces from which Child ('07b) obtained a complete hydranth.

In a fourth series the anlage of the proximal row of tentacles was just visible as a faint red area. The length of the piece removed was .6 mm. The time between the operations was forty hours. The following table gives the results from thirty pieces.

SERIES 4 A

Complete hydranth	0
Distal tentacles	25
No regeneration	5
Total number	30

Here no complete hydranths developed, while twenty-five formed the distal row which was probably already laid down when the two pieces were separated.

In a fifth series the piece *A* was removed immediately. This time the tip cut off exactly equaled the average area of the entire anlage (Fig. 23, *P*).

SERIES 5 A

Complete hydranth	12
Distal tentacles	0
No regeneration	3
Total number	15

This table shows that the small piece if isolated before any of the processes preparatory to hydranth formation are begun, is not only capable of forming a complete hydranth, but in a large proportion of cases it does form the complete structure. The length of time between the operations and also the size of the tip removed are factors in determining the extent of later regeneration in the end of the piece.

The later behavior of the proximal piece (Fig. 20, *B*) is given in the following parallel series where the tip was removed before the ridges appeared.

SERIES 1 B

Old proximal row retained, new distal in front.....	10
New anlage	15
	—
Total number	25

It will be seen upon comparing this series with Series 1 A in each there were ten pieces that retained the original tentacles that had been laid down; these ten pieces were parts of the same piece before the second operation. The fifteen proximal pieces in Series 1 B which formed entirely new primordia, developed on their original tips five complete hydranths, while ten died without any further development.

SERIES 2 B

Old proximal row retained, new distal in front.....	9
New anlage	13
	—
Total number	22

Again it will be seen by comparing this series with Series 2 A that some of the proximal and distal pieces retained the original anlage which had been started before the second operation, the larger number, however, developed a new anlage. Series 3 and 5 B will not be given as the pieces developed new hydranths in the usual way.

In the fourth series, the second operation was postponed until after the proximal ridges were discernible, but the distal had not yet appeared.

SERIES 4 B

Old proximal row retained, new distal in front.....	22
New anlage.....	8
	—
Total number	30

The method of completion of the hydranth on the proximal piece (*B*) when the original row persisted, in Series 1 and 2 was that described by Driesch as the "Ersatzanlagemodus," a new distal row developed in front of the proximal row before the hydranth emerged. That in Series 4 was Driesch's "Regenerationsmodus" where the hydranth emerged first, and later a new row of distal tentacles developed. I agree with Child ('07c) that there is no essential difference in the two methods.

CONCLUSIONS

These experiments prove that before the ridges have become visible, changes have taken place in the tip of the stem that render it when isolated incapable of producing a complete hydranth. These changes however do not take place until several hours after the wound has closed. If the tip is removed shortly after closure of the wound, even as long as twenty-four hours after the first operation, it is still possible for the isolated tip to form a complete hydranth. After the proximal tentacle anlage has appeared the distal piece when isolated, even if the tentacle ridges are not visible, does not form a complete hydranth. Instead the distal tentacles and mouth develop.

7 REVERSING THE DISTAL ROW OF TENTACLES

In earlier experiments ('00) I have shown that when the tip of the oral end of a long piece is cut off, reversed, and grafted back again at once, that the new hydranth develops in exactly the same region as it would have if the piece had not been removed. The distal row of tentacles appears in the small piece and the proximal row in the longer one. In the following experiment the tip was not removed until after the distal row of tentacles had appeared (Fig. 20). The piece *A* was then removed and grafted in

the reverse direction on the proximal piece (Fig. 24). This brought the distal tentacles into a position which was slightly different from their normal one. The space in front of them, i. e., between them and the end was greater than before. In a large number of experiments the pieces united, the tentacles completed themselves, and the normal hydranth emerged. In about 10 per cent of the grafts, a most interesting result was observed. The original distal tentacle ridges remained as distinct bands, while in front of them at the cut surface, a new row of distal tentacles and hypostome developed, the hydranth finally emerged with the stripes running from the base of the new distal tentacles to the proximal ones (Fig. 25). No reproductive organs formed on these pieces. The bands persisted until the hydranths dropped off. This serves to demonstrate that the small distal piece A when connected with the proximal piece is capable of developing new structures after the tentacles have been laid down. It also shows that the "red stuff" which is seen in the ridges, is not used again when a new row of tentacles is laid down. Stevens ('02) and Child ('07c) have observed in the proximal piece, after separation of the two ridges, that the "red stuff" which was originally in the proximal row forms a mass at the end of the stem, and when the hydranth is completed the mass is ejected.

8 REMOVAL OF THE ENTIRE PRIMORDIUM

Driesch ('02) found when he removed the early anlage of the hydranth by a cut just below the proximal row, that in a large number of cases the ridges disappeared, and a new anlage developed, the latter being much reduced in length. Thus the length of the "reparation area" bore a definite relation to the length of the piece. Child ('07a) has also shown that there is a reduction in length of the primordia in short pieces, but the reduction in length is not proportional to the reduction in the length of the piece.

I have made a series of experiments in which long pieces were cut from the stems of different individuals, and after the tentacle anlage appeared, the distal end of the piece was removed by a cut below the proximal tentacles (Fig. 26, X). I hoped to find that at

a fixed distance below the primordium the stimulus of the cut would result in the formation of a short aboral hydranth while the oral ridges were fading out and the new short ones were appearing. The results were as follows: When the space below exactly equaled the length of the primordium (Fig. 26) the hydranth continued to develop without any sign of delay caused by the cut. No hydranth developed on the aboral end for this region became the stalk. When the space below the primordium was equal to one-half the length of the primordium the result was the same. The cut was then made close to the base of the proximal row of tentacles. In about one-third of the pieces the original anlage disappeared and a new one much shorter than the first appeared. No aboral hydranths formed on these pieces.

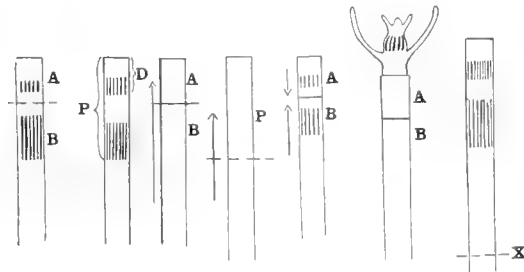


FIG. 20 FIG. 21 FIG. 22 FIG. 23 FIG. 24 FIG. 25 FIG. 26

9 THE INFLUENCE OF THE CONCENTRATION OF THE SEA-WATER

Loeb's earlier work ('92) on *Tubularia* brought to light the fact that the concentration of the sea-water is a definite factor in the regeneration of new hydranths. He found after testing various strengths, that sea-water diluted to 66 per cent was the optimum strength for growth. If the solution was weaker or stronger the growth was retarded. Snyder ('05) has also tested various strengths of sea-water, and has found that in *Tubularia crocea* when the sea-water was diluted not only was greater growth observed but a larger number of aboral hydranths developed.

My own experiments confirm those of Loeb and Snyder, but

some of the results which I obtained indicate that the great increase in size of the hydranths and the rapidity of their formation in dilute sea-water is due to something more than the difference in osmotic pressure.

The concentration of the sea-water in the Bay of Naples is estimated at 3.8 per cent. If this water is diluted to 2.5 per cent, growth is increased. Herbst ('04) found that artificial sea-water of the same strength as that of the Bay of Naples was more favorable for growth of sea-urchin larvæ than normal sea-water. I followed Herbst's formula and made a solution of artificial sea-water. I found that growth in this solution was more rapid and also that the hydranths formed in this solution were larger than usual, and lived longer. When the artificial solution was diluted with distilled water, the result was very different from that obtained from diluted water from the Bay. There was no increase in growth or in rate of regeneration. The results showed that the solution was not as favorable for growth as normal sea-water. I concluded from this that osmosis could not be the only factor in determining the increase in growth. It is not altogether improbable that organic substances in the Bay of Naples exert a retarding effect on growth. When these are excluded by preparing the pure artificial sea-water their retarding influence is abolished, and the growth which we consider so unusual is really no more than the normal rate under optimum conditions. This would explain why diluting artificial sea-water does not produce the same result as diluting that which comes directly from the Bay of Naples.

Child ('07c) has also made a study of the effect of diluting the sea-water. He concludes that the diluted medium increases the energy of the processes which involve hydranth formation. Since I made my experiments with dilute sea-water Child's work has been published. As my results are practically the same as his I shall omit a description of the experiments.

SUMMARY

1 When two individuals are grafted together changes at once take place in the region of the graft. These changes may not be

visible externally, but they exert some influence on the rest of the pieces, whether they result in the formation of new structures or not.

2 When the aboral end of a piece of the stem of *Tubularia* is stimulated through grafting to produce a hydranth before the oral end, the change in the polarity is not lasting for when a second set of hydranths develop, the piece assumes its original polarity.

3 When a short and a long piece are grafted together the influence of the longer piece is shown only when the new hydranth comes from the two pieces, a row of tentacles forming in each.

4 Short pieces grafted together in any direction usually develop a hydranth on one of the free ends only, or first on one end and later on the other. The result is the same whether the currents in the two pieces are continuous or not.

5 If the tip is removed from the end of a stem on which a new hydranth has just begun to develop, before the ridges are visible, the small piece is capable of forming a complete hydranth. If the tip is removed after the ridges are laid down, the piece develops one row of tentacles. The proximal piece completes itself distally by forming new tentacles on its tip, either before or after emerging from the perisarc. If the tip is removed before the primordia appear the proximal piece usually forms new primordia without delay.

6 If, after the appearance of the primordia, the piece in which the distal row of tentacles develops is reversed and grafted back on the proximal row, the hydranth completes itself in the normal manner. Sometimes the distal piece develops a new row of distal tentacles in front of the original red ridges which persist after the hydranth emerges.

7 When the entire primordium is removed by a cut just below the base of the proximal tentacles, the ridges frequently fade out and a new primordium develops which is much shorter than the original one. A cut 2 to 3 mm. below the primordium does not affect its later development. Such pieces do not form aboral hydranths.

8 Diluting normal sea-water in which pieces of *Tubularia* are placed, increases the rate of growth and the percentage of new

hydranths formed. Artificial sea-water has the same effect, but when the artificial sea-water is diluted these favorable results do not follow.

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A STUDY OF THE GERM CELLS OF CERTAIN DIPTERA, WITH REFERENCE TO THE HETERO- CHROMOSOMES AND THE PHENOMENA OF SYNAPSIS

BY

N. M. STEVENS

WITH FOUR PLATES

INTRODUCTION

In connection with previous work on the spermatogenesis of the Coleoptera ('05, '06), the germ cells of the common fruit-fly, *Drosophila ampelophila*, were examined in the autumn of 1906. The difficulties encountered in handling this material led to the study of the spermatogenesis of several other flies. The results will be presented in accordance with the following scheme:

Calyptrate Muscidæ.

Muscinæ.

- 1 *Musca domestica*.
- 2 *Calliphora vomitoria*.
- 3 *Lucilia cæsar*.

Sarcophaginæ.

- 4 *Sarcophaga sarracinæ*.

Anthomyiinæ.

- 5 *Phorbia brassica*.

Acalyptrate muscidæ.

- 6 *Scatophaga pallida*.
- 7 *Tetanocera sparsa*.
- 8 *Drosophila ampelophila*.

Syrphidæ.

- 9 *Eristalis tenax*.

METHODS

With this material it was found that the best results could be obtained from fresh tissue mounted in Schneider's aceto-carmin.

The testes (or ovaries) of adult flies were dissected out in physiological salt solution and immediately transferred to a drop of aceto-carmin on a slide. The cover-glass was pressed down with a needle to break the capsule of the testis and spread the cells. All excess of stain was removed with filter paper, and after ten or fifteen minutes, the preparation was sealed with vaseline. Such preparations may be studied to the best advantage after twenty-four hours, as the chromatin gradually acquires a deeper tint. They remain in good condition for several days, but are, of course, not permanent. The method has several advantages besides that of enabling one to examine a large amount of material in a limited time. The aceto-carmin fixes and stains instantly without the shrinkage incident to the usual treatment with fixing fluids, alcohols, xylol and paraffin, necessary in order to obtain sections. Then, one is able to study the whole cell with all of the chromosomes present and uncut, which is an obvious advantage for work of this kind. The chromatin stains much more deeply than any other cell element, but the achromatic structures are not always well brought out, and they have been omitted from most of the figures, as this investigation is concerned primarily with the heterochromosomes and the method of synapsis. In favorable preparations of this kind, with good light, it is possible to get as accurate camera drawings as from sections stained with iron hæmatoxylin.

RESULTS OF INVESTIGATION

I *Musca domestica*

In many respects the spermatogenesis of this fly resembles that of *Tenebrio molitor* (Stevens '05), *Odontata dorsalis* (Stevens '06) and the other Coleoptera which have an unequal pair of heterochromosomes. There are, however, no synapsis, synizesis or spireme stages in the spermatocytes, nor are tetrads ever formed.

In the prophase of spermatogonial mitoses one finds five pairs of long slender chromosomes, the members of each pair either lying parallel to each other or twisted together (Fig. 1). The members of the additional unequal pair are usually separate

(Fig. 1, h_1 and h_2). Apparently a side-to-side pairing or conjugation of homologous chromosomes, with the possible exception of the unequal pair, occurs preliminary to each spermatogonial mitosis. The twelve chromosomes separate, and each divides longitudinally in metakinesis. Whether they pair again in the telophase or not until the prophase of another cell-division is not evident.

The heterochromosomes remain condensed and are found side by side during the whole growth stage, while the other chromosomes pass into a more or less diffuse condition (Fig. 2). In the prophase of the first spermatocyte mitosis there are five thick V-shaped chromosomes and a pear-shaped mass of chromatin which in metakinesis proves to be the unequal pair of heterochromosomes (Fig. 3). The V-shaped chromosomes all divide longitudinally and the larger and smaller heterochromosomes separate as seen in Fig. 4. In the interval between the first and second divisions a nuclear membrane forms, but the chromosomes do not change greatly. Figs. 5 and 6 show the two kinds of daughter nuclei, one containing the larger, the other the smaller heterochromosome. In the second spermatocyte mitosis the V-shaped chromosomes again divide longitudinally and the heterochromosomes divide as shown in Figs. 7 and 8, so that in all stages they are clearly distinguishable from the ordinary chromosomes. The resulting spermatozoa fall into two equal classes, dimorphic as to the heterochromosomes, as in similar cases among the Hemiptera and Coleoptera. In most of the flies studied there was no difficulty in finding oögonia in which the number and relative size of the chromosomes could be determined. Only one such was found in *Musca*, that shown in Fig. 9. Here a part of the chromosomes are still paired; others have separated, but the members of each pair of ordinary chromosomes are not far apart; while the two equal heterochromosomes are on opposite sides of the group (h). Here again we have what may be regarded as a partial synapsis of homologous chromosomes. The relation of the heterochromosomes in the two sexes is the same as in many of the Coleoptera (Stevens '05 and '06) and the Hemiptera heteroptera (Wilson '05 and '06), an unequal pair (large and small) in

the male and an equal pair of large heterochromosomes in the female. An egg which is fertilized by a spermatozoön containing the smaller heterochromosome produces a male, while one which unites with a spermatozoön containing the larger heterochromosome produces a female.

Although there is no distinct synapsis stage visible in the development of the spermatocytes of *Musca domestica*, the method of synapsis is without doubt indicated by the side-to-side pairing of chromosomes of equal length in the prophases of both spermatogonial and oögonial mitoses. The final synapsis is a closer union of the homologous chromosomes, and the first spermatocyte division separates the members of each pair instead of dividing each chromosome as in the spermatogonia.

2 *Calliphora vomitoria*

The chromosomes in this species are similar to those in *Musca domestica*. Both members of the unequal pair of heterochromosomes are smaller, as may be seen in a spermatogonial metaphase (Fig. 10). Pairing of homologous chromosomes is also evident here. In the growth stage (Fig. 11) the heterochromosomes are associated with a plasmosome as in many species of *Coleoptera*. Two views of the metaphase of the first spermatocyte mitosis are shown in Figs. 12 and 13, and an anaphase in Fig. 14. Two metaphases and an anaphase of the second division appear in Figs. 15, 16 and 17. The equal pair of heterochromosomes in the female is clearly shown in two oögonial metaphases (Figs. 18 and 19). In this case we have further evidence of the side-to-side pairing of homologous chromosomes in the spermatogonia and oögonia.

3 *Lucilia cæsar*

Only a few specimens of this species were captured and the series of stages is incomplete. No spermatogonial or oögonial metaphases were found. In the growth stage a pair of *m*-chromosomes is present with an enormous heterochromosome bivalent

(Fig. 20). The metaphase of the first spermatocyte division is shown in Figs. 21 and 22, and prophases of the two kinds of second spermatocytes in Figs. 23 and 24. The spermatozoa would evidently be dimorphic as in the other species.

4 *Sarcophaga sarracinia*

The three species of Diptera whose spermatogenesis has already been described belong to the sub-family Muscinae, while *Sarcophaga* is a member of the sub-family Sarcophaginae. The number of chromosomes in *Sarcophaga* is the same as in the other species, 12 somatic and 6 reduced, and the heterochromosomes closely resemble those in *Calliphora*. The spermatogonial plate (Fig. 25) shows the 12 chromosomes paired, but separated ready for metakinesis, and one chromosome shows the division line. In the growth stage (Fig. 26) the pair of heterochromosomes comes out clearly in the midst of diffuse and irregular masses of faintly stained chromatin. In these flies the ordinary chromosomes become much branched or diffusely granular in the growth stage but do not unite to form a spireme of even width as in so many forms. Whether or not they unite end-to-end at any stage before or after synapsis I cannot say. A prophase and an equatorial plate of the first spermatocyte mitosis may be seen in Figs. 27 and 28, and the metaphase and anaphase in Figs. 29, 30 and 31. The polar views of the metaphase of the second mitosis (Figs. 32 and 33) of course show dimorphism as to the heterochromosomes (h_1 , h_2). Equal division of all of the chromosomes follows as in the three preceding species. Figs. 34 and 35 were drawn from adjacent oögonia in metaphase to show the close longitudinal pairing of the chromosomes and their later separation before metakinesis. The equal heterochromosomes are usually found together in the middle of the plate and each one is evidently equivalent in size to the larger heterochromosome of the spermatogonia and spermatocytes (Figs. 25 to 33). Fig. 36 is from an ovarian follicle cell. The four figures, 25, 34, 35 and 36 show the pairing of homologous chromosomes in spermatogonia, oögonia and somatic cells.

5 *Phorbia brassica*

Only one male of *Phorbia* was obtained and only four stages drawn; but these indicate precisely the same conditions as in the other species examined. *Phorbia* belongs to the sub-family Anthomyiinae. Fig. 37, a growth stage; 38, a prophase; 39, a metaphase; and 40, an anaphase, show clearly the presence of an unequal pair of heterochromosomes resembling those of *Musca domestica*.

6 *Scatophaga pallida*7 *Tetanocera sparsa*

The chromosomes of *Scatophaga* and *Tetanocera* resemble each other so closely in number, form and behavior that they will be considered together. Fig. 41 is a spermatogonial prophase of *Scatophaga*; and Figs. 42 and 43, spermatogonial prophase and metaphase of *Tetanocera*. All show equally paired ordinary or V-shaped chromosomes and unequally paired heterochromosomes. Figs. 44 and 45 are prophase and metaphase of the first spermatocyte of *Scatophaga*, Figs. 46 and 47 the corresponding stages for *Tetanocera*. In both species it will be seen that there is a close resemblance between the paired condition of the chromosomes in the prophases of a spermatogonial division and of a first spermatocyte mitosis. In general the chromosomes were larger in the spermatogonia (Figs. 41, 42, 43) than in the spermatocytes (Figs. 44, 45, 46, 47), but frequently prophases of spermatocyte mitoses could be certainly identified as such only by the metaphases in the same cyst and the growth stages in the neighboring cysts. The only actual observable difference between the synaptic condition in the spermatocytes and the spermatogonia is the behavior of the pairs in the following mitosis: in the spermatogonia the members of the pairs separate in metaphase (Fig. 43), and each divides in metakinesis; while in the spermatocytes the members of each pair remain closely associated in metaphase (Figs. 45 and 47) and separate in metakinesis (Fig. 48), but do not divide until the

second spermatocyte mitosis, though they frequently show the preparatory split in the anaphase (Fig. 49). We have here an unusually clear demonstration of the essential facts of synapsis and reduction, together with the rather unusual phenomenon of conjugation of homologous chromosomes in cells outside the sphere of maturation. Prophases of the second spermatocyte mitosis in *Scatophaga* appear in Figs. 50 and 51, and metaphases in *Tetanocera* in Figs. 52 and 53. An oögonial prophase and anaphase are given in Figs. 54 and 55, and a late prophase for *Tetanocera* in Fig. 56.

These two species as well as the one following belong to the Acalyptrate Muscidæ.

8 *Drosophila ampelophila*

Drosophila has been placed at the end of the list of Muscidæ because of the peculiarities which occur in the behavior of its chromosomes and the difficulties which have been encountered in their interpretation. While in *Sarcophaga* all the stages necessary for a description of the behavior of the heterochromosomes of both sexes were found in the course of a few hours' work on perhaps ten or twelve preparations, satisfactory results in the case of *Drosophila* have been obtained only after a prolonged study extending over more than a year and involving the dissection and examination of some two thousand individuals. Sectioning the material has never given satisfactory results. Hermann's platino-osmic solution and Worcester's formal-sublimate gave the best fixation, but the division stages are so scattering that permanent preparations, even if good fixation were secured, seemed less practical than the aceto-carminé method, which is much quicker and gives clearer pictures of the mitotic phenomena when they are present.

Spermatogonial mitoses are not abundant, and cells in which perfectly clear equatorial plates can be studied are exceedingly rare. The chromosomes in prophase are paired and twisted together in such a manner that it has been impossible to make an intelligible drawing of them in an early prophase. In Fig. 57, a

late prophase, two small spherical chromosomes and four larger elongated ones are distinctly paired while the members of the unequal pair (h_1 , h_2) are separated. For a long time it was impossible to be sure that an unequal pair was present, as foreshortening in the case of one chromosome (h_2) was possible, but recently a comparatively large number of good spermatogonial plates has been secured in which the inequality in length of one pair is clearly demonstrated. No case has been found in which the members of this pair appeared to be equal. Figs. 58, 59 and 60 show exceptionally clear cases, and Fig. 61 shows a peculiar folding of the chromosome h_1 , whose significance may be apparent as we proceed to consider the unequal heterochromosome bivalent of the first spermatocyte.

In *Drosophila* the heterochromosomes cannot be demonstrated in the growth stages of the first spermatocyte. In some sections from Hermann material stained with thionin the plasmosome (p) and some of the chromosomes appeared as in Fig. 62 in cysts adjacent to the spermatogonial cysts. In later growth stages nothing definite, except the immense plasmosome, can be made out in regard to the contents of the nucleus. The earliest prophase of division is the appearance of the chromatin massed together, usually on one side of the nucleus, while the plasmosome may be in the middle or on one side of the nucleus (Fig. 63). In aceto-carminic preparations the chromosomes first appear in early prophase, scattered through the nucleus, faintly stained and irregular in outline (Fig. 64). The plasmosome may be broken up at this time or it may appear intact in the spindle. Figs. 65 and 66 are later prophases in which the chromosomes are completely condensed. The unequal heterochromosomes are h_1 and h_2 . Fig. 67 shows the three equal bivalents, and the unequal heterochromosome pair in its simplest form, in the metaphase of the first spermatocyte mitosis. Fig. 68 shows slight modifications of this form from other cells of the same cyst. The most common form of this pair is seen in Figs. 69 and 70, where there are two equal V-shaped elements and a third portion (\bar{x}) which in many cases looks like a separate element, and for a time the group was thought to be trivalent; i. e., made up of two equal V-shaped

chromosomes and a smaller odd chromosome. This belief was strengthened by the appearance of many metaphases and anaphases (Figs. 70, 71, 72) where the third portion of the figure (x) seemed to be on the point of separating from the V-shaped element next to it. This opinion was not confirmed however by the composition of the spermatogonial or oögonial equatorial plates, nor was it possible to demonstrate with certainty a separate element corresponding to x in the polar plates of the first spermatocyte mitosis or in the second spermatocyte. Fig. 72 is one of several cases where the portion x seemed to be separated from the two other elements of the group, but the separation must have been only apparent, for one much oftener finds an anaphase like Fig. 73 where the separation of the heterochromosome group into two unequal parts is certain (h_1 , h_2). Sometimes the anaphase is like Fig. 74, where more or less spherical masses replace the usual V's of the heterochromosome group. Often all of the chromosomes except the smallest pair show in the metaphase that they are elongated and V-shaped (Fig. 75), and in late anaphases (Fig. 76) the elements of the two largest bivalents are usually divided and the daughter chromosomes separated, often crossed. Both here and in the second spermatocytes it is often difficult or impossible to distinguish the heterochromosomes from the others. In the telophase the chromatin forms a dense mass which loses none of its staining quality and is soon resolved into the already divided chromosomes of the second spermatocytes (Figs. 77, 78, 79). A greater or less degree of elongation together with twisting and fore-shortening makes it impossible to measure or even estimate with any accuracy the relative length of the chromosomes, so as to distinguish the two classes of second spermatocytes as to size of heterochromosomes. Figs. 78 and 79 are two equatorial plates from the same cyst where the corresponding chromosomes are probably $a-a$, $b-b$, and h_1-h_2 . All of the chromosomes divide in this mitosis.

The oögonial metaphases are perfectly clear, and four equal pairs of chromosomes are always present (Figs. 80, 81, 82). In the metaphase they are usually grouped in pairs, and in the prophase they are closely approximated and twisted. In fact this

prereductional pairing of homologous chromosomes was first noticed in the oögonia and ovarian follicle cells of *Drosophila*. An attempt was made to ascertain whether such a pairing occurs in embryonic cells. Very little evidence was obtained. In the prophase of one mitosis paired chromosomes were found (Fig. 83). Fig. 84 is the equatorial plate of a segmentation stage. In both cases the pairs appeared to be equal.

9 *Eristalis tenax*

A considerable number of these flies were captured on some late blooming mustard plants in October. The material was in exceptionally favorable condition, and a complete series of drawings was obtained. The outer wall, or capsule, of the testis was thinner and more permeable to fixing fluids than in most of the other species studied and it was therefore possible to work with both sections and aceto-carmin preparations. This fly belongs to the family Syrphidæ, but the chromosomes in most respects resemble those of the Muscidæ. The heterochromosome bivalent is different in form from that of any of the Muscidæ described above; it however consists of a larger and a smaller component united in a somewhat different way from the corresponding elements in *Drosophila*.

Among the spermatocytes, several follicle cells were found in mitosis; the chromosomes of one such is shown in Fig. 85. The spermatogonial chromosomes are paired in prophase but separate and form a flat plate in the metaphase as seen in Fig. 86, where the two heterochromosomes (h_1 , h_2) are conspicuously unequal in size. In this form there is a distinct synizesis stage, as shown in Fig. 87, from a section of material fixed with Gilson's mercurio-nitric fluid and stained with thionin. The cysts in which this stage occurs border upon the spermatogonial region of the testis. The outline of the chromosomes is visible and in the next stage the chromosomes are distinctly bivalents. Later they become more diffuse, but do not appear to form an even spireme at any stage. Fig. 89 is a growth stage, showing the heterochromosome group (h), a pair of m -chromosomes and the other chro-

mosomes in a loosely branched condition. Fig. 90 is an early prophase in which the heterochromosome pair is very compact and deeply stained, while the other chromosomes are granular and denser in some parts than in others. A later prophase (Fig. 91), from a section, shows the heterochromosome pair assuming the cross-shape which we find in the later metaphase. Fig. 92 is a polar view of the equatorial plate of the first spermatocyte; and Figs. 93 and 94, side views of the spindle to show the cross-shaped heterochromosome bivalent in two positions. Here the cross (Fig. 94), instead of having opposite arms equal, as in cross-shaped tetrads composed of equal elements, has one of the vertical arms longer. It is evident from Figs. 93 and 95 that the longer arm is the smaller heterochromosome, while the remainder of the cross is the larger member of the pair. The larger element is folded in the same manner as in *Drosophila* (Figs. 66 and 67) but the smaller element is attached by one end instead of by the middle as in *Drosophila*. The second spermatocyte mitosis proceeds as in the other forms and presents nothing of especial interest. Dimorphism of the spermatozoa is foreshadowed by the first spermatocyte anaphases (Figs. 96 and 97). In the female the clearest figures were obtained from ovarian follicle cells (Figs. 98 and 99). The pairs are equal and comparison with the spermatogonial chromosome group (Fig. 86) indicates that the equal heterochromosome pair is one of the two longest.

The general results for the nine species of flies are the same; i. e., an unequal pair of heterochromosomes in the male leading to dimorphism of the spermatozoa, and a corresponding equal pair in the female, each equivalent to the larger heterochromosome of the male: also a prereductional pairing of homologous chromosomes in the prophase of mitosis in spermatogonia, oögonia, and ovarian follicle cells.

DISCUSSION

1 Sex Determination

So far as I know there is no published work on the heterochromosomes of the Diptera. The literature on the heterochromo-

somes in other orders of insects has recently been so fully discussed in a paper by A. M. Boring ('07) that it seems hardly necessary to go into the subject exhaustively here. The dimorphism of the spermatozoa resulting from the maturation of the male germ cells of the nine species of Diptera considered in this paper is of the same character as that described by the author for 36 species of Coleoptera (see note, p. 49, Stevens '06), and by Wilson ('05 and '06) for several species of Hemiptera heteroptera. The dimorphism is brought about by the presence in the spermatogonia and spermatocytes of an unequal pair of heterochromosomes, while in large numbers of other insects such dimorphism is due to the presence of an odd chromosome in the male germ cells. These flies have proved to be exceptionally favorable material for demonstrating the occurrence in the female germ cells and somatic cells of a pair of chromosomes, each equivalent to the larger heterochromosome of the male.

Here, as in similar cases previously described, it is perfectly clear that an egg fertilized by a spermatozoön containing the smaller heterochromosome produces a male, while one fertilized by a spermatozoön containing the larger heterochromosome develops into a female. The material does not, however, throw any further light on the question whether the dimorphic spermatozoa are themselves in some way instrumental in determining sex in these insects; or whether sex is a character borne by the heterochromosomes and segregated in the maturation of the germ cells of each sex. If the latter supposition is true, sex is probably determined by the dominant heterochromosome of the egg, and fertilization is selective as has been shown in previous papers (Wilson '05, '06; Stevens '06, p. 54; Nowlin '06; Boring '07).

The only hope of determining whether sex is a Mendelian character seems at present to lie in breeding experiments with forms that may be shown by cytological study to be favorable. It is probable that in some cases at least, other characters may be so correlated with sex that their behavior in heredity may throw light on the sex question.

As to the proportion of sexes in these flies, a few figures may be given for *Drosophila ampelophila*. In the autumn and winter

of 1906-07, *Drosophila* was bred in the laboratory on two kinds of food, grapes and bananas. As the flies were dissected for the cytological work, a record was kept of the numbers of each sex; 1551 were so recorded. Of these 759, or 48.94 per cent were males; 792, or 51.06 per cent females. The records of the grape-fed and the banana-fed flies were kept separately. The total number of grape-fed flies dissected between November 1 and March 19 was 787, 404 or 51.33 per cent being males, and 383 or 48.67 per cent females. The banana-fed flies between October 30 and December 3 numbered 764, 355 or 46.47 per cent males, and 409 or 53.53 per cent females. In the total number there were 2.02 per cent more females than males, in the grape-fed 2.66 per cent more males than females, and in the banana-fed 7.06 per cent more females than males. These differences are probably not significant, but if sex is a Mendelian character, the numbers for the two sexes should of course be equal unless food produces some discriminating effect on the development of either individuals or eggs of the different sexes. It has always been a noticeable fact that the banana-fed flies were larger and more robust than those fed on grapes; this however applies to both sexes. In mass cultures it is not possible to tell whether failure of many of one sex or the other to reach the adult stage in different cultures might account for the discrepancies in numbers observed with the two kinds of food.

Castle and his co-workers ('06 p. 772) found the sexes about equal in three families of the sixth inbred generation of a grape-fed series, and the remarks which follow the table indicate that they regard the normal proportion as near equality.

Monkhaus' results on sex in *Drosophila* seem not yet to be in print, except for a brief report in the Year Book of the Carnegie Institution.

An attempt was made to ascertain the normal proportion of the sexes for the adults of *Musca domestica*. When caught by hand 58.33 per cent were females, but when a wire trap baited with sugar or molasses was used, only 46.53 per cent were females. The results need no comment.

Cuénot states that the normal proportion of males to females

in *Lucilia cæsar*, *Calliphora vomitoria* and *Sarcophaga carnaria* is approximately equal, and his experiments show that neither amount nor kind of food given to the larvæ has any marked effect on the proportion of the sexes in the first or second generation, but here as elsewhere in such experiments the number of eggs that did not hatch is not noted, and this may be the critical point. It is evident that more experiments are needed in which the fate of all of the eggs of isolated pairs of flies is determined.

2 *Synapsis*

In the spermatogenesis of most insects synapsis involves an end-to-end union of homologous chromosomes, and tetrads of various forms are commonly found in the prophase of the first spermatocytes. In these flies no tetrads have been observed and as a rule nothing comparable to the synizesis, bouquet or spireme stages of other forms is apparent. In these respects the germ cells of the Diptera resemble the oögonia of *sagitta* (Stevens '03 and '05) and the male and female germ cells of the aphids (Stevens '05 and '06). In the oögonia of *Sagitta* the chromosomes pair side-to-side in an early stage, while in the spermatogonia of the aphids the pairing occurs as a prophase of the first spermatocyte mitosis. The indications are that in the flies the chromosomes are already paired side-to-side at the beginning of the growth stage (Figs. 87 and 88), but the pairs do not appear to unite end-to-end to form a spireme. In some cases the members of the pairs are perfectly fused in the prophase of the first spermatocyte (Figs. 3 and 27); in others the bivalents are clearly such in both prophase and metaphase (Figs. 44 to 46). The first spermatocyte division is without doubt reductional for both ordinary chromosomes and heterochromosomes.

Perhaps the most interesting point in the whole study is the pairing of the chromosomes in cells somewhat removed from the sphere of the reduction process. This was first noticed in the oögonia of *Drosophila*, and was also found to occur in the ovarian follicle cells, the spermatogonia and some embryonic cells. This is not an occasional phenomenon, but one which belongs to every

oögonial and spermatogonial mitosis. In many cases the pro-phases of spermatogonia and first spermatocytes resemble each other very closely, the members of each pair being twisted together in both. In the spermatocyte we get complete synapsis and reduction; in the spermatogonium only a foreshadowing of reduction, and abundant proof that synapsis is here a side-to-side pairing of homologous chromosomes, and the first spermatocyte division a separation of univalent chromosomes, and not a longitudinal or quantitative division of two chromosomes united end-to-end. The relation of the heterochromosomes to each other in synapsis varies greatly with differences in form and size.

One is tempted to suggest that if homologous maternal and paternal chromosomes in the same cell ever exert any influence on each other, such that it is manifest in the heredity of the offspring, there is more opportunity for such influence in these flies than in cases where pairing of homologous chromosomes occurs but once in a generation. Possibly experiments in cross-breeding of flies may bring out some interesting facts in heredity.

NOTE. A preliminary statement in regard to the chromosomes of *Drosophila* was made at the International Congress of Zoölogists in Boston, August 21, 1907. The question as to whether an odd chromosome or an unequal pair of heterochromosomes was present in the male cells was then unsettled.

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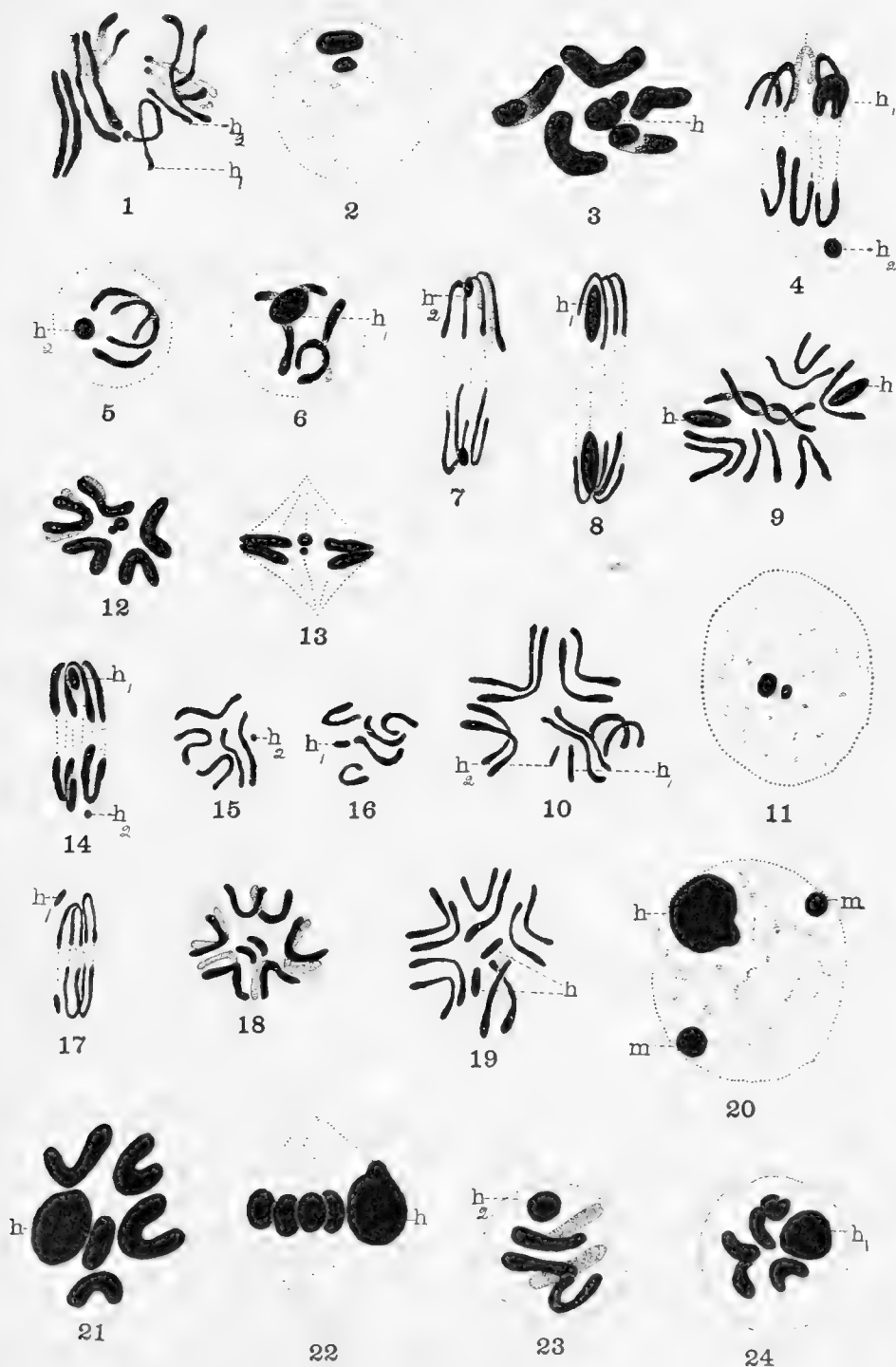


PLATE II

Sarcophaga sarracenia

- Fig. 25 Spermatogonium, metaphase.
- Fig. 26 First spermatocyte, growth stage.
- Fig. 27 First spermatocyte, prophase.
- Fig. 28 First spermatocyte metaphase.
- Fig. 29 First spermatocyte, metakinesis.
- Figs. 30 and 31 First spermatocyte, anaphase.
- Figs. 32 and 33 Second spermatocyte, metaphase.
- Figs. 34 and 35 Oögonia, metaphase.
- Fig. 36 Ovarian follicle cell, metaphase.

Phorbia brassica

- Fig. 37 First spermatocyte, growth stage.
- Fig. 38 First spermatocyte, prophase.
- Fig. 39 First spermatocyte, metaphase.
- Fig. 40 First spermatocyte, anaphase.

Scatophaga pallida and *Tetanocera sparsa*

- Fig. 41 Scatophaga, spermatogonium, prophase.
- Fig. 42 Tetanocera, spermatogonium, prophase.
- Fig. 43 Tetanocera, spermatogonium, metaphase.
- Fig. 44 Scatophaga, first spermatocyte, prophase.
- Fig. 45 Scatophaga, first spermatocyte, metaphase.

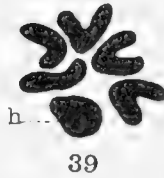
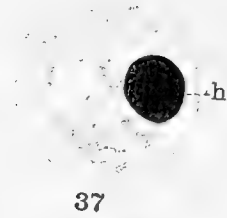
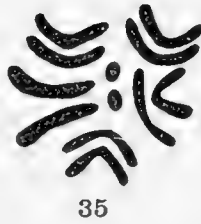
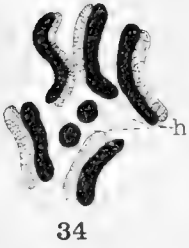
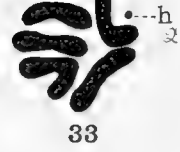
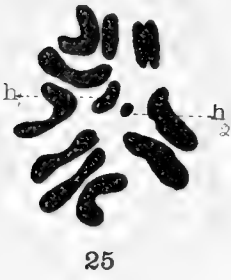


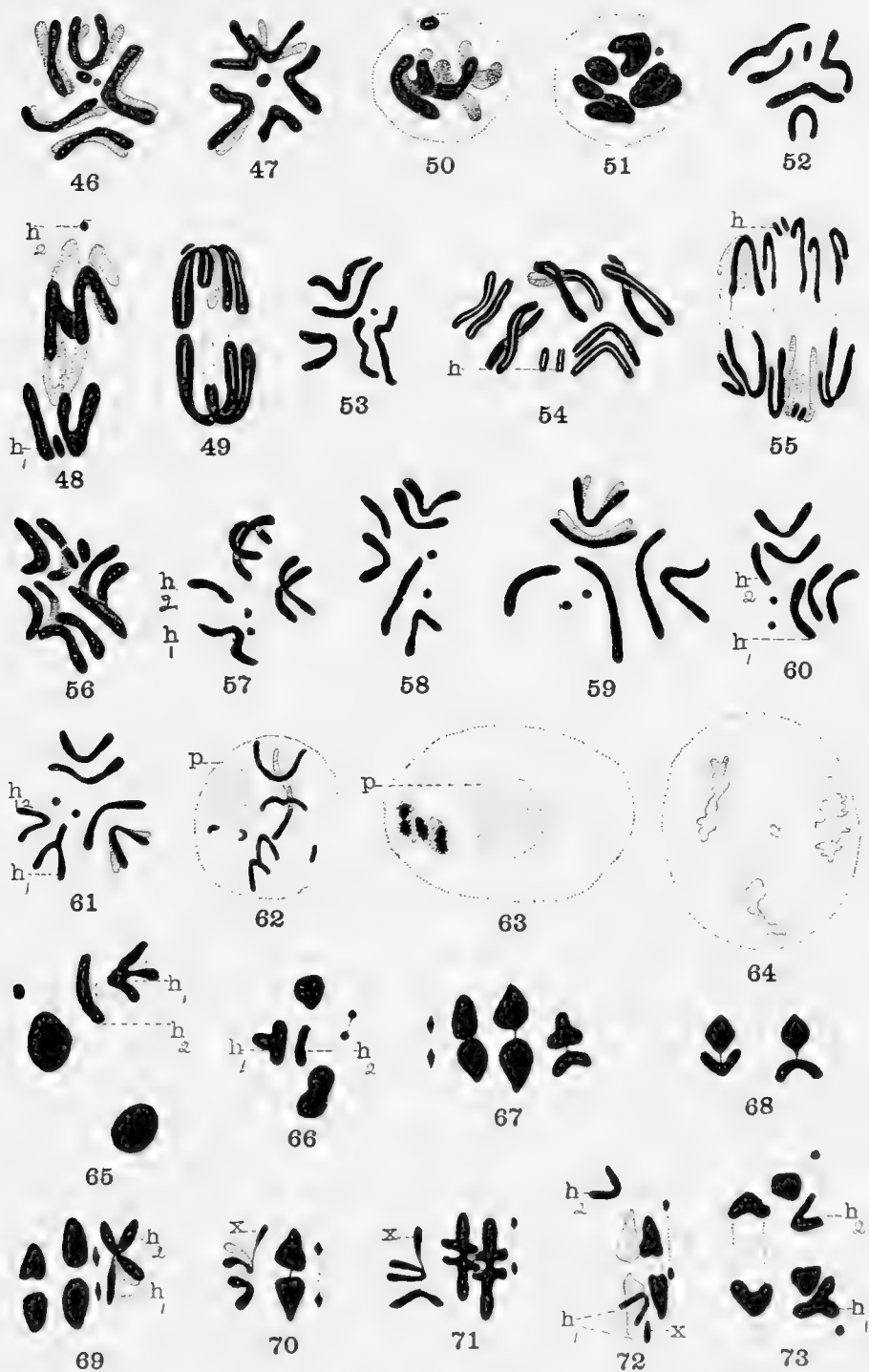
PLATE III

Scatophaga and *Tetanocera* (continued)

- Fig. 46 *Tetanocera*, first spermatocyte, prophase.
Fig. 47 *Tetanocera*, first spermatocyte, metaphase.
Fig. 48 *Scatophaga*, first spermatocyte, anaphase.
Fig. 49 *Scatophaga*, first spermatocyte, anaphase.
Figs. 50 and 51 *Scatophaga*, second spermatocyte, prophase.
Figs. 52 and 53 *Tetanocera*, second spermatocyte, metaphase.
Fig. 54 *Scatophaga*, oögonium, prophase.
Fig. 55 *Scatophaga*, oögonium, anaphase.
Fig. 56 *Tetanocera*, oögonium, prophase.

Drosophila ampelophila

- Fig. 57 Spermatogonium, late prophase.
Figs. 58-61 Spermatogonia, metaphase.
Fig. 62 First spermatocyte, early growth stage.
Fig. 63 First spermatocyte, very early prophase.
Fig. 64 First spermatocyte, prophase.
Figs. 65 and 66 First spermatocyte, late prophase.
Fig. 67 First spermatocyte, metaphase.
Fig. 68 Heterochromosome pairs.
Figs. 69-71 First spermatocyte, metaphase.
Figs. 72 and 73 First spermatocyte, anaphase.



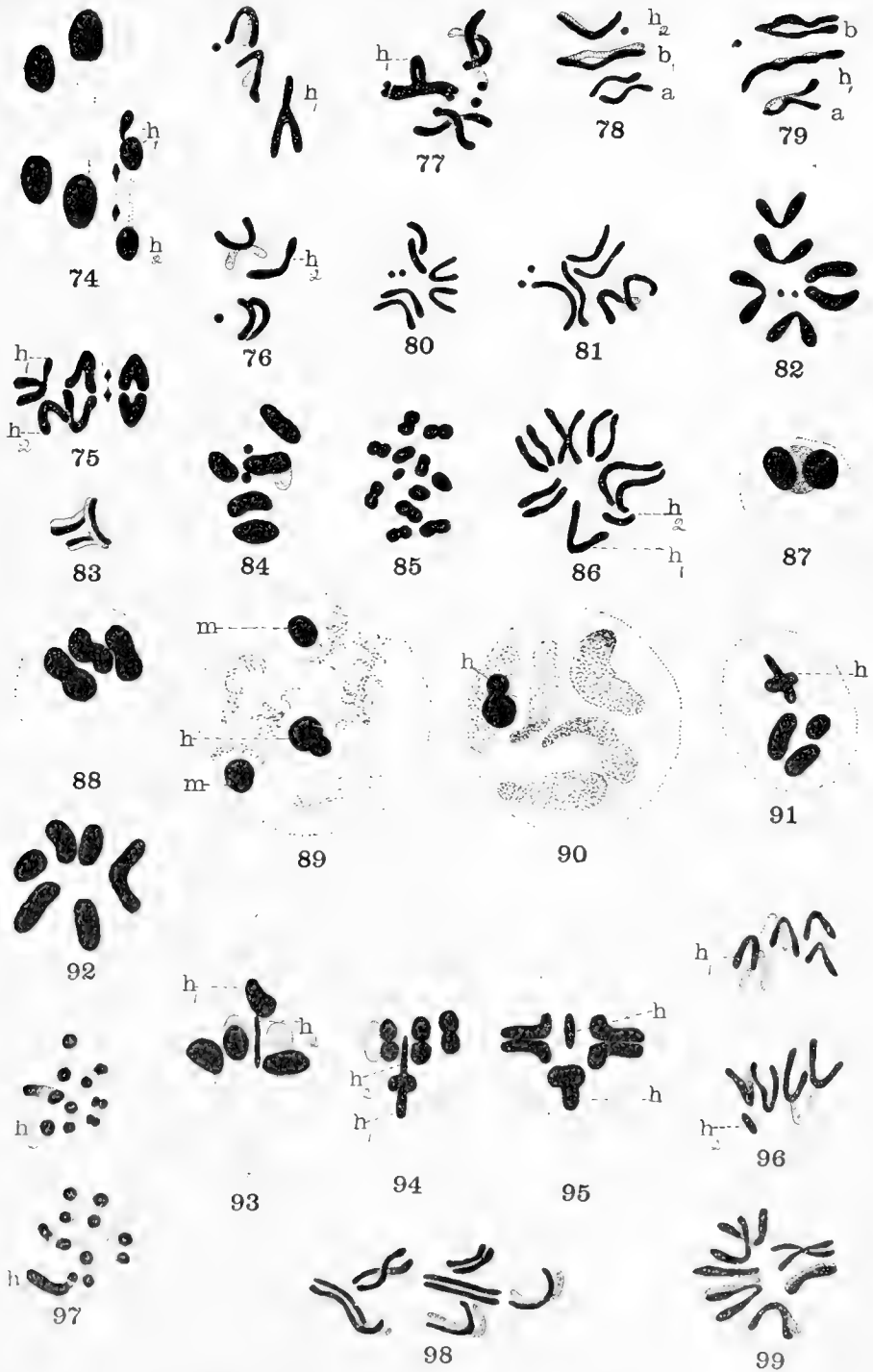
PALTE IV

Drosophila (continued)

- Fig. 74 First spermatocyte, anaphase.
- Fig. 75 First spermatocyte, metaphase.
- Fig. 76 First spermatocyte, anaphase.
- Figs. 77-79 Second spermatocyte, metaphase.
- Figs. 80-82 Oögonia, metaphase.
- Fig. 83 Chromosomes from embryonic cell.
- Fig. 84 Chromosomes from segmentation stage.

Eristalis tenax

- Fig. 85 Chromosomes of follicle cell of the testis.
- Fig. 86 Spermatogonium, metaphase.
- Fig. 87 First spermatocyte, synizesis stage.
- Fig. 88 First spermatocyte, growth stage immediately following synizesis stage.
- Fig. 89 First spermatocyte, later growth stage.
- Fig. 90 First spermatocyte, early prophase.
- Fig. 91 First spermatocyte, late prophase.
- Figs. 92-95 First spermatocyte, metaphase.
- Figs. 96 and 97 First spermatocyte, anaphase.
- Figs. 98 and 99 Chromosomes of ovarian follicle cells, prophase and metaphase



MOMENTARY ELEVATION OF TEMPERATURE AS A MEANS OF PRODUCING ARTIFICIAL PARTHE- NOGENESIS IN STARFISH EGGS AND THE CON- DITIONS OF ITS ACTION

BY

RALPH S. LILLIE

I INTRODUCTION

Exposure of mature eggs of *Asterias forbesii* to the influence of cold sea-water (about 4° to 7°) for somewhat prolonged periods (1 to 7 hours) was first shown by Greeley,¹ at Wood's Hole in 1901, to be followed by cleavage and production of larvæ on return to normal temperatures. Greeley also experimented with temperatures higher than the normal, exposing eggs (taken from the same dishes as those used for experiments with cold) to temperatures of 31° to 37° for similar periods of time (1 to 7 hours); but the results of this treatment were purely negative, the eggs merely absorbing water and undergoing a change which he described as liquefaction. He concluded, somewhat sweepingly, that "segmentation of the starfish egg cannot be produced by raising the temperature of the sea-water." He found later (summer of 1902) that temperature was an important factor in the production of parthenogenesis by hypertonic solutions,² the time of exposure decreasing (within a certain range of temperatures) as the temperature rose, a result confirmed by Lyon³ at Naples in the fall of 1902 for species of *Strongylocentrotus* and *Arbacia*. But elevation of temperature alone, unaccompanied by other treatment, remained ineffective; moreover, "at 30° it was found impossible to produce artificial parthenogenesis in *Asterias* or *Arbacia* with any of the solutions used." In the earlier paper Greeley had

¹ Greeley: American Journal of Physiology, vi, p. 296, 1902.

² Greeley: Biological Bulletin, iv, p. 129, 1903.

³ Lyon: American Journal of Physiology, ix, p. 308, 1903.

treated with incredulity Delage's account of successful experiments with higher temperatures, ascribing the results to the effects of agitation and not of simple elevation of temperature. In Greeley's own experiments "when great care was exercised in handling the eggs not a single segmentation was produced." The criticism, however, was ill-founded, for it was clear from Delage's papers⁴ that his eggs were exposed to the high temperatures at a time—namely, early maturation when agitation is quite ineffective in producing parthenogenesis. It is not until the eggs have been mature for some time that this result appears;⁵ while warming, as Delage expressly affirms, is most effective during early maturation stages. In Greeley's second paper he again cites Delage's experiments, but without comment. Evidently his intention was to return to the subject.

Since the appearance of Delage's papers in 1901 there seems to have been little further investigation of the influence of rise of temperature in exciting development of unfertilized eggs. The theoretical possibility that development could thus be induced was incidentally adverted to by Loeb⁶ some years later: if the spermatozoon acts by introducing positive catalysers into the egg, thus accelerating the chemical processes on which the initiation of development depends, a similar acceleration with similar consequences ought to follow simple elevation of temperature. Loeb has also more recently emphasized the importance of the temperature factor in the production of parthenogenesis by the use of hypertonic solutions.⁷ But no further experimental contributions have appeared toward the solution of the question whether—and under just what conditions—elevation of temperature can in itself initiate the development of unfertilized eggs. The *a priori* probability that such would be found to be the case must have seemed strong when the high temperature-coefficient of the acceleration of chemical processes was considered: a five or sixfold acceleration of at least certain of the reactions occurring in the egg-substance would

⁴ Delage: Comptes rendus, cxxxiii, p. 348, 1901; Archives de zoologie expérimentale et générale, 3me Sér., ix, p. 285, 1901.

⁵ Mathews: American Journal of Physiology, vi, p. 142, 1902.

⁶ J. Loeb: University of California Publications, Physiology, vol. ii, p. 158, 1905.

⁷ J. Loeb: Biochemische Zeitschrift, vol. i, p. 183, 1906.

follow warming to 35° or 40°; and a fundamental change in the properties of the system and possibly a removal of the conditions impeding spontaneous development might reasonably be expected to result from such treatment.

The failure of investigators in this country to obtain parthenogenesis by elevation of temperature appears the less accountable since Delage's descriptions are at least sufficiently definite to have suggested a procedure quite different from the one which was actually employed and proved ineffectual. Thus Delage writes⁸ "La température peut, à elle seul, surtout appliquée brusquement à un stade critique, dont il va être question, déterminer la parthénogénèse chez *Asterias*." This critical stage is described as the time (approximately) at which the nuclear membrane of the germinal vesicle disintegrates allowing the nuclear contents to enter the cytoplasm; this event determines the time at which "merogonic" fertilization becomes possible, and also artificial parthenogenesis by heat: "at this moment the eggs of *Asterias* can be made to develop parthenogenetically by simple immersion in water warmed to 30° to 33°."⁹ The lack of exactitude in this description consists chiefly in the failure to assign any definite limit of time to the action of the warm sea-water. As will be seen below, this is a matter of importance, since too long and too brief exposures alike fail to produce the desired effect and lead simply to abnormal changes resulting in the early disintegration of the egg. It is clear, however, that the eggs in Delage's experiments were warmed for only a short period; in fact, he recommends placing the eggs in warm sea-water contained in small vessels (cuvettes) which may be rapidly cooled in running water.¹⁰ In Greeley's experiments the eggs (1) were allowed to mature—a necessary condition for the production of parthenogenesis by cold, action of acids, agitation, or hypertonic solutions, but one which precludes the possibility of development by simple warming (as will be seen below); and (2) were exposed to the high temperatures for periods of an hour or more; whereas exposure to 35° for 60 or 70 seconds

⁸ Delage: *Comptes rendus*, cxxxi, p. 348, 1901.

⁹ Delage: *Comptes rendus*, *loc. cit.*, p. 348.

¹⁰ Delage: *Archives de zoologie expérimentale et générale*, *loc. cit.*, p. 309.

is sufficient, at the proper time during the maturation period, to produce development. It is not surprising that the eggs failed to develop under these conditions. The general outcome of Greeley's own work on the influence of temperature changes on protoplasm appears to have led him to doubt the possibility of producing parthenogenesis by elevation of temperature. He had found that cold, by inducing loss of water, exercises on protoplasm an action similar to that of a hypertonic solution, which was already known to produce parthenogenesis; and it must have seemed to him scarcely possible that warmth, which affects the protoplasm in a precisely opposite manner from cold, could have the same influence on the developmental process. It is also evident that his experiments on the action of high temperatures in inducing parthenogenesis were less complete than those made with cold; evidently his studies of the influence of temperature-conditions on development were cut short while they were yet unfinished.

II EXPERIMENTAL

My own experiments were begun in the summer of 1906, at a time when I was unaware that Delage had succeeded in producing development by this means. The idea with which the study was begun was that possibly a slight change in the aggregation-state of certain of the protoplasmic colloids might be a determining condition of development, and that such a change might be induced by a momentary heating of the eggs. Heat coagulation produced by momentary heating followed by rapid cooling was, according to Corin and Ansiaux, a reversible process.¹¹ Such a slight and reversible coagulation might conceivably without injuring the egg so change the state of the egg substance as to cause development to be resumed. It soon became evident, however, that even transitory exposure to temperatures of 45° to 50°, the lowest at which heat coagulation could be expected, was rapidly injurious, inducing breakdown of the eggs without any developmental changes. On the other hand, brief exposure to temperatures of 35° to 38°—in

¹¹ Corin and Ansiaux: *Bulletin de l'académie royale de Belgique*, xxi, p. 345, 1891. The results of Corin and Ansiaux have since been rendered doubtful by Pauli: *Beiträge zur chemischen Physiologie und Pathologie*, x, p. 53, 1907.

general the optimum for enzyme action—gave extremely promising indications. The remainder of the investigation was then devoted to determining the influence of such temperatures acting for various brief periods.

In the following experiments the eggs were exposed for brief periods (varying from a few seconds to several minutes) to the action of sea-water previously warmed to a definite temperature. The procedure employed is as follows: the eggs are transferred at a known period after removal from the animal to a small beaker in which a thermometer is placed; sea-water at a temperature slightly above that selected for the particular experiment (e. g., 35°) is then added rapidly to the small beaker in quantity sufficient to bring the temperature to the desired point; this temperature is maintained constant during the definite time-period of the experiment by partly immersing the small beaker, whenever necessary, in a larger vessel of water at somewhat higher than the experimental temperature. After the lapse of the selected time-period (e. g., 70 seconds) the contents of the small beaker are suddenly transferred to a large volume of sea-water at normal temperature contained in a finger-bowl. The temperature of the eggs is thus suddenly reduced again to the normal. It may safely be assumed, when one considers the small volume of each egg and the correspondingly large surface for thermal interchange with the medium, that during at least the greater part of the period of immersion in the warm sea-water the eggs have themselves been at the same temperature as the medium. The agitation involved in the two transfers is unavoidable with this procedure; but at the stages with which I have worked—mostly early maturation—mechanical shock is in itself ineffective in causing development. Mere transfer from one dish to another produces no visible result. The effects observed are therefore to be ascribed wholly, or at least in chief part, to the change in the thermal conditions prevailing in the egg-system.

Experiments with Arbacia Eggs

The results with sea-urchin eggs have been almost entirely negative so far as concerns production of development by momen-

tary elevation of temperature. In the earliest experiments, eggs were exposed for a few seconds to temperatures supposedly high enough to cause partial coagulation of a portion of the colloidal constituents of the protoplasm. Temperatures of 45° , 50° , 55° , and 60° were allowed to act for periods ranging from 5 to 60 seconds. No noteworthy changes followed such treatment; swelling and disintegration resulted from exposure for even brief periods to the higher temperatures. A few eggs showed membranes similar to fertilization membranes after exposure to 45° for a few seconds, and occasionally some cleavages were found. The great majority of eggs so treated died without showing any developmental change.

Treatment that resulted favorably with *Asterias* eggs also gave imperfect or negative results with *Arbacia*. Eggs were exposed to 35° , 37.5° , and 40° , for periods ranging from five seconds to two minutes. In the most favorable experiments a few eggs showed membranes and irregular cleavages; but development never proceeded beyond a stage of a few cells, and the great majority of eggs always remained apparently unaffected. I have also attempted to induce cleavage in unfertilized *Arbacia* eggs after the artificial production of a fertilization membrane by the method introduced by Loeb, viz: treatment for one to two minutes with a mixture of 3 cc. $\frac{N}{10}$ acetic acid and 50 cc. sea-water. Eggs so treated become, as in the case of *Strongylocentrotus* investigated so thoroughly by Loeb, far more susceptible to the development-inducing action of hypertonic sea-water; but the results after warming to 35° for periods of 20, 30, 40, 60 and 90 seconds, within 10 to 15 minutes after membrane-formation, were in no observable respect different from those obtained with the same eggs after simple treatment with acidulated sea-water without warming. A certain proportion of such eggs always undergo cleavage, usually irregular, but development rarely proceeds farther than an early stage of a few cells.

A striking phenomenon, which I have frequently observed in sea-urchin eggs treated in the above manner with acidulated sea-water, seems entitled to special mention here, namely, the appearance of active amœboid movements of the egg-protoplasm, at times surprisingly energetic in character. The movement appears most

active about three or four hours after treatment with the acidulated sea-water. The following record will illustrate:

July 15, 1907, 12:37 p.m. Unfertilized sea-urchin eggs were placed in a mixture of 50 cc. sea-water + 3 cc. $\frac{N}{10}$ acetic acid; one portion (A) was transferred to normal sea-water after one minute, a second (B) after 1 m. 30 s. At 4:30 p. m., lot A showed numerous irregularly shaped eggs in which active amœboid movement was in progress. In many eggs the movement was so energetic that the actual contractions of the cell-surface and the protrusion of pseudopodia were plainly visible; many even exhibited an active crawling or squirming movement, suggestive of sluggish muscular contractions. In many eggs small portions of the surface protoplasm were constricted off—small beadlike protuberances like polar bodies being especially numerous. Transitions between irregular amœboid masses and distinct though irregular cleavage stages were not uncommon; the latter also showed continual and active changes of form. Lot B showed essentially similar conditions. The temperature of the water in the dishes was 25°.

This observation seems interesting on account of the unusually active nature of the amœboid movements. The assumption of irregular amœboid forms by various eggs is familiar to most experimentalists,¹² and is especially frequent in starfish eggs. But active crawling movements of the above kind have, so far as I am aware, not hitherto been described in these eggs. The theoretical interest of the phenomenon consists chiefly in the very clear indication which it affords that the form-changes in cleavage are of essentially the same nature and due to the same conditions as are the ordinary amœboid movements of cells; these last, as may be inferred from the closeness with which they may be artificially simulated, are almost certainly due—at least as regards their main features—to local (possibly electrically conditioned) changes of surface tension. The above transitional condition between amœboid movement and cleavage supports strongly the view that the change of form in normal cell-division is also due to surface-tension changes, which differ from those causing amœboid movements only in the very regular and symmetrical distribution of the areas of lowered surface tension.

Experiments with Starfish Eggs

A Conditions of Formation of Fertilization-membrane

Exposure to temperatures of 45° and higher caused mature starfish eggs to become coarse and opaque within 20 minutes or less.

¹² Especially energetic amœboid movements are seen in abnormally developing parthenogenetic eggs of *Chaetopterus*; cf. F. R. Lillie, *Archiv f. Entwicklungsmechanik*, xiv, p. 487, 1902.

No membrane was formed. In one series of experiments, eggs in early maturation stages (at which time membranes are most readily formed) formed in some instances membranes on exposure to 45° for 15 seconds; exposure to the same temperature for 30 seconds was followed by breakdown without membrane-formation. Temperatures of 45° and higher are thus rapidly destructive, as in the case of sea-urchin eggs; but very brief exposures may produce some of the effects (as membrane-formation) of more favorable conditions.

Temperatures of 40° and lower were then tried. The earliest visible effect of brief warming at such temperatures is the formation of the fertilization membrane. The production of this membrane appears to be associated with the removal of certain hindrances to further development (p. 385), and accordingly it may be regarded as the first visible sign of developmental changes in the egg. The structure is produced with remarkable ease by momentary exposure of eggs to the action of warm sea-water; yet it is significant that temperatures above a certain maximum (*ca.* 45°), acting for more than a few seconds, fail to cause its production. Apparently some ferment-action, rather than the direct effect of the heat, is concerned. It also fails to be produced at 30° unless possibly the exposure is very prolonged. I have made few observations with temperatures lower than 35° . The temperature relations of this phenomenon ought perhaps to be more thoroughly investigated.

The following table summarizes the results of three series of experiments covering a considerable range of temperatures. They illustrate very typically some of the conditions of membrane-production in starfish eggs.

TABLE I

Series I. July 30, 1906

Temperature degrees	Time of exposure seconds	RESULT	
		A Eggs warmed before separation of first polar bodies	B Same eggs warmed four hours after removal from animal
30	15	No membranes formed	
	30	No membranes formed	
35	15	No membranes	No membranes formed
	30	Membranes in more than half	A few membranes
40	15	All form membranes	Most form membranes
	30	All form membranes	Almost all form membranes
45	15	All form membranes	Almost all form membranes
	30	None form membranes; eggs soon disintegrate	No membranes; eggs soon disintegrate
50	15	No membranes; early disintegration	
	30	No membranes; early disintegration	

Series II. August 1, 1906

Temperature degrees	Exposure	RESULT	
		A Eggs warmed during maturation process	B Warmed 2½ hours after completion of maturation
33	15 s.	No membranes	Practically no membranes
	30 s.	No membranes	Practically no membranes
	60 s.	Almost all form membranes	A few membranes
	2 m.	Almost all form membranes	A few membranes
35	15 s.	A few imperfect membranes	Considerable number membranes
	30 s.	Most form membranes	Practically all form membranes
	1 and 2 m.	All form membranes	All form membranes
37.5	5 s.	A few imperfect membranes	A few membranes
	15 s.	Practically all form membranes	Practically all form membranes
	30 s.	All form membranes	Practically all form membranes
	1 m.	All form membranes	Practically all form membranes
40	5 s.	Practically all form membranes	
	15 and 30 s. and 1 m.	All form membranes	

TABLE I—Continued

Series III. August 6, 1906. Eggs warmed during maturation process

Temperature degrees	Exposure	RESULT
33	30 and 60 s.	No membranes
	2 m.	Fair number of membranes
34	30 s.	No membranes
	1 and 2 m.	All form membranes
35	30 s.	Most form membranes
	1 and 2 m.	All form membranes
36	30 s.	Almost all form membranes
	1 and 2 m.	All form membranes
37	15, 30, 60 s.	All form membranes
38	15, 30, 60 s.	All form membranes

In general the above observations may be regarded as typical, though I have found some variability in the readiness with which eggs from different animals form membranes. But with starfish eggs in the early maturation period membrane-formation rarely or never fails if eggs are exposed to temperatures between 33° and 40° for the periods indicated as optimal in the above table. The result is remarkably constant, even if the subsequent cleavage and development should prove abnormal or should altogether fail. The facility with which the membrane is produced varies also in eggs from the same animal at different intervals after removal; in general the early maturation stages, before the first polar body is separated, are most favorable; after the completion of maturation, membrane-production is less regular and constant, and more prolonged exposures to the high temperature are necessary. This change is possibly to be correlated with the change in susceptibility to parthenogenetic development under this form of treatment, which also diminishes after maturation is completed, as I shall describe later.

The minimum time of exposure necessary for membrane-production is shown by the above experiments to decrease rapidly with rise of temperature until a certain limit is reached. At 33° exposure must be prolonged to two minutes; at 34° the minimum lies somewhere between 30 and 60 seconds; at 35° between 15 and 30

seconds; at 37.5° between 5 and 15 seconds, and at 40° momentary exposure (5 seconds) produces membranes in practically all eggs. These temperature-relations point to an underlying process that undergoes unusually rapid acceleration with rise of temperature, until a certain optimum is reached (apparently in the neighborhood of 40°), after which heat acts unfavorably. Exposure to 45° for 30 seconds fails, as seen above, to produce membranes and acts destructively on the eggs, although briefer exposure (15 seconds) may be effective.

The actual separation of the membrane may be readily studied. Within 10 to 15 minutes after return to normal sea-water it appears as a wavy or crenated layer adhering closely to the egg-surface; this layer gradually detaches itself as the sea-water enters the space next the cell-surface, and with the resulting distension the inequalities disappear; after 20 to 25 minutes (at 20° to 22°) the membrane is uniform and normal in appearance, though still very near the egg-surface. The process may be characterized as secretory in nature, and it appears to be dependent on a partial solution of the superficial lipoid layer of the egg; this is indicated by its ready production through the action of the various fatty acids and fat-solvents. The above temperature-relations appear to indicate, in the case of production by warming, a dependence on some enzyme action. If a simple solution of certain substances at higher temperatures were the determining condition, the high temperature-coefficient of acceleration, as well as the failure of temperatures above 45° so to act, would be unintelligible. On the other hand, the assumption of dependence on some process accelerated by an enzyme with an optimum temperature of 38° to 40° , and rapidly destroyed at 45° , would account for the above relations. Certain hydrolytic cleavages may be concerned, possibly a saponification resulting in a partial solution of the surface layer; the production of the same effect by the action of fat-solvents or alkalis becomes readily intelligible on such an assumption.

An important significance has been ascribed by Loeb to the process of membrane-production in sea-urchin eggs. After membrane-formation, however induced, the condition of the egg is altered in such a manner that relatively brief exposure to hyper-

tonic or hyperalkaline solutions is sufficient to produce normal development.¹³ Even without such after-treatment, eggs in which membranes have been produced frequently cleave and under certain conditions may form blastulæ; usually, however, such eggs undergo breakdown or cytolysis within a few hours. Since this change, as well as the cleavage, is dependent on the presence of free oxygen, the inference is drawn that in some manner, possibly by removal of anticatalytic substances, membrane-formation leads to an acceleration of oxidation processes in the egg; these if properly directed—as in consequence of normal or parthenogenetic fertilization—lead to cell division and development; otherwise they result in the destruction of the egg. Membrane-formation has thus an important significance in development.

My own observations on the starfish egg in some respects support this conclusion, though they can scarcely be said to do so uniformly. That the process of membrane-formation is not essential to cleavage has been known for some time; Loeb's early studies in artificial parthenogenesis supply instances of cleavage without formation of fertilization membranes, and he cites other instances in a later paper.¹⁴ It is also possible artificially to suppress membrane-formation without destroying the possibility of cleavage in the following manner: Eggs were placed 15 minutes after removal in $\frac{M}{2000}$ KCN solution in sea-water, and remained here 20 hours; they were then washed in normal sea-water and warmed to 35° for 70 seconds; these eggs formed no membranes although a considerable proportion underwent irregular cleavage. On another occasion the same suppression of membrane-formation without prevention of cleavage was observed in eggs exposed to $\frac{M}{2000}$ KCN for only two hours. Although cleavage is thus to a certain degree independent of membrane-formation, nevertheless normal cleavage and development certainly do appear to be facilitated by the separation of the membrane. In the above cited experiments development stopped short at an early stage, and I have never found eggs to develop to an advanced stage under this form of treatment without the formation of a membrane. On the other hand, when-

¹³ Loeb: *loc. cit.*, also *Archiv für die gesammte Physiologie*, cxviii, pp. 181 and 572, 1907.

¹⁴ Loeb: *University of California Publications, Physiology*, vol. ii, p. 153, 1905.

ever mature eggs are treated in such a way as to form fertilization membranes—whatever method is used—a certain proportion are always found to undergo cleavage. Another observation that I have frequently made appears to favor the idea that there is a correlation between membrane-formation and the acceleration of oxidation processes in the egg. I have found uniformly that the coagulation and disintegration which follow when mature eggs are allowed to remain for some hours in normal oxygen-containing sea-water, occur much more rapidly in eggs that have formed membranes than in those that remain without this structure. Thus, warming eggs during early maturation to 35° for 25 or 30 seconds induces membrane-formation in a large proportion—usually about one-half—but not in all of the eggs; practically all of the eggs so treated die at an early stage; if they are examined after 18 hours, those with membranes are invariably found to be in an advanced state of disintegration, the entire space enclosed by the membrane being filled with a mass of loose granular detritus; those without membranes, on the other hand, although coagulated and opaque, are still compact and undisintegrated. The same contrast between eggs with and without membranes in the rate and character of the disintegration is seen when the membranes are formed by the action of ether or a fatty acid. This result, which I have found with perfect uniformity throughout the present investigation, shows that eggs which have formed membranes, yet without undergoing normal development, exhibit less resistance to the disintegrative action of the post-maturation oxidative changes than do those lacking these formations. It is possible that the greater cytoplasmic activity of the eggs with membranes (as shown by the production of pseudopodia and the irregular cleavages and other form-changes) may facilitate the disintegrative process; the effect may also conceivably be dependent, at least in part, on simple mechanical conditions: the change in the closely adhering surface-layer of the unaltered egg, due to the removal of the membrane-forming substance, would probably facilitate the action of any disintegrative agency. One might suggest that the mechanical resistance to surface-changes, including cleavage, is lessened by the formation of a membrane, and that the significance of the

process in facilitating developmental changes may possibly lie here.

The membrane is readily formed by brief exposure, during or after the maturation stage, to the action of sea-water containing xylol or ether; and such eggs show the typical irregular form-changes and cleavages; I have however not yet obtained free-swimming blastulæ from eggs thus treated. Treatment for one or two minutes with a solution of 3 cc. $\frac{N}{10}$ acetic acid in 50 cc. sea-water produces perfect membranes, and I have frequently obtained a small proportion of swimming larvæ from eggs so treated.¹⁵ The effect must be regarded as due to the lipolytic action of the fatty acid and not as a general effect of acidity (or increased concentration of hydrogen ions) since mineral acids— H_2SO_4 and HNO_3 —used similarly fail to produce the least sign of a membrane.¹⁶

The ability of mature eggs to form membranes as a result of momentary warming shows a certain periodical variation, as will be shown in more detail later (cf. pp. 400, 403). In general the dissolution of the germinal vesicle is an important condition, although immature eggs may form perfectly typical membranes under certain conditions (p. 407). Again, as already shown, membrane-formation by heating becomes more difficult after maturation is complete. On the other hand, treatment with a fatty acid appears to produce membranes with equal readiness at any time after maturation has begun. Thus I have subjected successive portions of a single lot of eggs to the action of the above acetic acid solution at 10 minute intervals throughout the entire course of maturation (until the separation of the second polar body) and again an hour later, without finding any decided difference in effect at the different periods; a small proportion of blastulæ was obtained in every one of the ten experiments of the series except the first (treated 10 minutes after removal from animal). The largest proportion of larvæ was obtained from eggs treated previously to the separation

¹⁵ Compare Loeb: *loc. cit.*, and *Dynamics of Living Matter*, 1906, p. 172

¹⁶ Loeb (*loc. cit.*, cf. also *Dynamics of Living Matter*, p. 170) found the same difference between fatty and mineral acids. Lefevre, however, finds that in *Thalassema* mineral acids produce membranes with the same readiness as do fatty acids. Here apparently some other action than the directly lipolytic is involved. Cf. Lefevre: *Journal of Experimental Zoölogy*, vol. iv, p. 106, 1907.

of the first polar body; still, no such well-defined periodicity was found as with the experiments on the effects of warming (pp. 396, et seq.) The appearances indicate a difference in the conditions of the membrane-forming process—the acid acting by a simple lipolytic action on the surface layer, while the effect of heating depends on acceleration of an enzyme action, as already suggested. Variations in the quantity or in the condition of the enzyme would affect the results of warming without altering those due to the simple action of a fat solvent.

Later I shall give some further observations on membrane-formation in starfish eggs. The process certainly seems to be correlated with a change in the developmental capabilities of the egg. It does not however necessarily lead to an acceleration of the oxidations in the egg, as is shown by the fact that immature eggs in which membranes have been formed show no increased disposition to undergo the typical oxidative coagulation or cytolysis (p. 408); yet under certain conditions (after maturation has begun) such an accelerated oxidation does seem to result and to constitute an important condition of development, as already indicated. The experiments to be described later show, however, that only a small part of the effects of momentary warming can thus be accounted for. In the starfish egg, in fact, repression rather than acceleration of oxidations seems to be an important condition of the initiation of the developmental process, although this latter once begun naturally requires free oxygen for its continuance (p. 413, et seq.)

B Development of Eggs after Momentary Warming

Membrane-formation is followed after a more or less prolonged interval by a series of form-changes in the egg; these under favorable conditions take the form of regular cleavage. It must be regarded as significant that the most manifold and irregular changes of form may occur, with all gradations between the protrusion of pseudopodia and the assumption of various irregular uncleaved forms or the production of irregular and unequal cleavages and fragmentations on the one hand, and the normal process

of typically regular and equal cleavage on the other. The irregularities are extremely various, and it is difficult to assign any definite conditions to the production of any particular kind. They seem largely due to changes occurring in the cytoplasm independently of the nucleus; in other words, there is frequently a lack of correlation between nuclear and cytoplasmic activities in the warmed eggs; certain processes are initiated in both, sometimes leading to nuclear division independently of cytoplasmic division, at other times to the apparently independent assumption of irregular forms on the part of the cytoplasm, with the production of irregular pseudopodia, usually followed by subdivision of the cytoplasm into unequal cleavage cells or still smaller enucleate fragments. Such fragmentation is very typical of eggs that have been warmed for too prolonged periods; the formation of small bead-like protuberances which then separate from the rest of the cell-body is an especially frequent phenomenon. These conditions as a rule reach their height about three or four hours after warming, at a time when the first cleavages usually begin to appear in regularly dividing eggs.

The production of protoplasmic processes at times shows remarkable peculiarities, particularly in eggs derived from animals late in the season or otherwise unfavorable. The proportion of irregular form-changes is also greater in eggs warmed after maturation is complete (p. 402). A slightly prolonged warming often leads to the production of numerous long slender close-set pseudopodia of clear protoplasm, of a uniform length sometimes equal to that of the egg-radius, imparting a prickly or radiating appearance to the entire structure; irregular fusions may take place between these processes as in the pseudopodia of Foraminifera.¹⁷ These cytoplasmic activities seem to have little directly to do with nuclear influence; separated enucleate portions of protoplasm may also undergo irregular form-change or subdivide still further. Other instances of specific change of form in enucleate portions of eggs have been described by several observers. It seems clear that the

¹⁷ Such conditions seem frequent in abnormally developing parthenogenetic eggs; compare especially the accounts of F. R. Lillie for *Chaetopterus*, *loc. cit.*, p. 487; also of Lefevre for *Thalassema*, *loc. cit.*, p. 109.

cytoplasm possesses a certain formative capacity of its own;¹⁸ this under the above abnormal conditions may give rise to structures having very definite peculiarities, but quite foreign to the normal development.

Under favorable conditions a large proportion of eggs undergo regular cleavage and development. The following series (Table II) will illustrate; the eggs (all from a single lot) were exposed to temperatures of 35°, 36°, 37° and 38°, for varying brief periods during the early maturation period (between 20 and 45 minutes after removal from the animal, before separation of the first polar body). The susceptibility varies somewhat within this period; but, as will be shown later, warming may produce development at any time between the dissolution of the germinal vesicle and the formation of the first polar body (after which time it becomes increasingly difficult to incite development by this means). Within at least the greater part of the period of exposure covered by this series the susceptibility to development by warming varies relatively slightly, and the condition of the eggs may be regarded as essentially uniform throughout. Later experiments will be described in which the variation in susceptibility at different periods during maturation is itself made the subject of study (cf. pp. 396, et seq.)

Eggs from the same lot were treated in a precisely similar manner on the afternoon of the same day, from 2.36 to 3.06 p.m. All had matured in the typical manner. The result was quite different. Membrane-formation was less uniform and required a more prolonged exposure to the respective temperatures, and although in favorable experiments a considerable proportion of eggs underwent cleavage, mostly irregular, not a single swimming larva was obtained. This kind of experience has been uniform. I have never succeeded, after the completion of maturation, in bringing unfertilized eggs to the free-swimming stage. The eggs invariably either fail to cleave, or cleave more or less irregularly, usually after undergoing irregular form-changes, and die at an early stage.

¹⁸ Compare Wilson's account of the phenomena in the isolated enucleated polar lobe of *Dentalium*; cf. also the references in his paper to analogous phenomena in echinoderm eggs. Wilson: *Journal of Experimental Zoölogy*, vol. 1, p. 53, 1904.

TABLE II

August 8, 1906. Eggs were removed from the animal at 10:15 a.m., and treated as follows: Temperature of sea-water in the dishes, 23°

	Interval after removal from animal minutes (ca.)	Temperature and time of exposure		RESULT
		deg.	sec.	
1	20	35	30	No membranes formed. No cleavage
2	21	35	40	Ca. 50 per cent form membranes; many cleavages, mostly irregular; no blastulæ obtained
3	22	35	50	All form membranes; numerous regular cleavages; a few blastulæ obtained
4	24	35	60	All form membranes. Cleavage largely regular; blastulæ more numerous than in Experiment 3
5	25	35	70	All form membranes. Cleavage more regular and rapid than in Experiment 4; good proportion gastrulæ
6	27	35	80	Similar to 5; good proportion blastulæ and gastrulæ
7	29	36	15	Practically no membranes (one seen). No cleavage
8	29.5	35	20	All form membranes. Mostly irregular cleavage. No larvæ
9	30	36	30	Similar to 8
10	31	36	40	More favorable; large proportion regular cleavages and a fair number of blastulæ and gastrulæ
11	32	36	50	Somewhat more favorable than 10; a considerable number of blastulæ and gastrulæ
12	36	37	10	A few membranes formed; no cleavage found
13	37	37	15	Most form membranes; cleavage mostly irregular; no blastulæ obtained
14	38	37	20	All form membranes; mostly irregular cleavages, a few regular; no larvæ found
15	39	37	30	More favorable than 14; good proportion regular cleavages; large number blastulæ and gastrulæ obtained, and a few good Bipinnariæ
16	40	37	40	Fewer regular cleavages than in 15; a fair number of larvæ obtained
17	41	38	5	Hardly any membranes (2 or 3 seen). No cleavages found
18	41	38	10	Almost all form membranes. Cleavage irregular or incomplete. No larvæ
19	42	38	15	All form membranes and cleavage is less irregular than in 18. Some regular cleavages, and a few blastulæ and gastrulæ obtained
20	43	38	20	Large proportion of irregular cleavages and a fair proportion give swimming blastulæ and gastrulæ; a few reach Bipinnaria stage

The time of early maturation (before the separation of the first polar body), is apparently a critical period for the production of this type of parthenogenetic development. The same has been found true by Delage.¹⁹

A similar series of experiments on July 24, 1907, with the three temperatures 35°, 36° and 37° and a somewhat different range of exposures gave in general the same result, summarized in Table III.

TABLE III

July 24, 1907. Eggs were removed at 2:15 p.m., and treated as follows

	Interval after removal minutes (ca.)	Temperature and exposure		RESULT
		deg.	sec.	
1	30	35	60	Good proportion of regular cleavages, and fair number blastulæ and gastrulæ
2	31	35	70	Cleavage more rapid and regular than in 1; large number active larvæ obtained
3	33	35	80	In general like Experiment 2: somewhat less favorable; numerous larvæ
4	34	35	90	Smaller proportion of regular cleavages and fewer larvæ than in Experiments 2 and 3
5	37	36	50	All form membranes, but cleavage is mostly irregular; no larvæ
6	38	36	40	Larger proportion cleavages than in 5, largely regular. No larvæ
7	40	36	50	Fair proportion of regular cleavages, fair number of blastulæ and gastrulæ obtained
8	42	36	60	Cleavage less regular and slower than in 7; good many blastulæ and gastrulæ
9	45	37	20	All maturing eggs form membranes, relatively few cleavages; no larvæ obtained
10	46	37	30	Large proportion of regular cleavages; fair number of blastulæ and gastrulæ obtained
11	47	37	40	Fewer cleavages, slower and less regular than in Experiment 10; eggs mostly adopt irregular shapes without cleaving; a few larvæ
12	48	37	50	Eggs form membranes and adopt irregular shapes with slender pseudopodia; no cleavages found. No larvæ

¹⁹ Delage: *loc. cit.*

From the above experiments it appears that the optimum time of exposure to the high temperatures is shorter the higher the temperature employed. In the above two series the best results were obtained at 35° with 70 seconds exposure, at 36° with 40 or 50 seconds, at 37° with 30 seconds, and at 38° with 20 seconds. In general the same relative favorability of different periods of exposure at different temperatures was found in several other similar series of experiments. The decrease in the optimum time of exposure with a given increase in the temperature is somewhat surprisingly rapid, the difference of three degrees between 35° and 38° reducing the optimum exposure from 70 to about 20 seconds. If the process in which the initiation of development depends is a purely chemical one, this would indicate an extraordinarily high temperature-coefficient of acceleration. The conditions in a heterogeneous system like protoplasm must, however, be recognized as peculiar; rise of temperature, in addition to accelerating the specific chemical processes (usually about threefold for a rise of 10°), has a certain effect (which under some conditions may be very considerable) in altering the surface of interaction between the colloidal bodies concerned and the other chemical substances reacting with them; increased subdivision of the colloidal particles following a rise of temperature would in itself accelerate the reaction; and the total acceleration would be measured by the product of this increase in the surface of interaction into the specific acceleration of the process itself through the rise of temperature; this compound value might exceed many times (as apparently in the case under consideration) the simple temperature-coefficient of acceleration of the purely chemical process. The results with membrane-production also indicate a very high temperature-coefficient. So far as regards development I have as yet made no special endeavor to determine the optimum periods of exposure at temperatures above and below those cited. The favorable periods for temperatures of 39° and 40° would undoubtedly be found very short, while at 34° and 33° exposures would be more prolonged. Temperatures so low as 30° would in all likelihood be found effective with sufficient time of exposure, as Delage's²⁰ results indicate.

²⁰ Delage: *loc. cit.*, p. 307.

The different temperatures do not however seem equally favorable, and more larvæ are obtained at 35° and 36° than at 37° and 38° ; in other words, with the higher temperatures warming seems more likely to produce abnormal results. My experience has been that temperatures of 35° , acting for somewhat longer than one minute, have usually given the best results. This is illustrated by the foregoing tables and again by the following four experiments. In this series eggs from a single animal were exposed to 35° for 60, 70, 80 and 90 seconds, respectively, at a period of 30 to 35 minutes after removal from the animal. The results were as follows:

1	35° 60 s.	A good proportion reach gastrula stage and a few form early Bipinnariæ
2	35° 70 s.	A large proportion reach advanced gastrula stage and a considerable number form early Bipinnariæ
3	35° 80 s.	Somewhat less favorable than Experiment 2; a considerable number form advanced gastrulæ, and a few early Bipinnariæ
4	35° 90 s.	Fewer larvæ obtained than in the above three experiments and none reach early Bipinnaria stage

An exposure of 70 seconds to 35° again proves most favorable. In all of the following experiments on the determination of the period of greatest susceptibility to this treatment I have accordingly employed uniformly an exposure to 35° for 70 seconds; such treatment if applied at a favorable period (best at some little time before the separation of the first polar body) almost invariably results in the production of a good and sometimes a high proportion of larvæ. There appears however to be some variation in the optimum period of exposure to a given temperature in eggs from different animals and at different seasons of the year. Thus on September 6, 1906, eggs were treated as follows during early maturation (17 to 37 minutes after removal): 35° for 70, 75, 80 and 85 seconds; 36° for 40, 45, 50, 55 and 60 seconds; 37° for 20, 25, 30 and 35 seconds. Cleavage was most nearly normal and a certain rather small proportion of larvæ was obtained with 35° for 85 seconds, 36° for 50, 55 and 60 seconds (the last best), and 37° for 30 and 35 seconds (both about equally good). With the other exposures the cleavage was slower and less regular and no swimming larvæ

resulted. Another portion of the same eggs was similarly treated in the afternoon about three hours after completion of maturation; a large proportion failed to form membranes, cleavage was either irregular or failed altogether, and not a single larva resulted. This series was less favorable than those tabulated above, and the optimum exposures were considerably more prolonged. The difference in the time of year may be a factor of importance; this, however, can only be determined by further experiment. On the whole, when normal eggs are used a given temperature has a quite well defined optimum period of exposure which can be determined with considerable accuracy. Since the temperature-coefficient of acceleration of a given process may afford valuable indications as to its essential nature, a more exact redetermination of the optimal periods of exposure through a greater range of temperatures may yield valuable results. I hope at some future time to make more extended and exact determinations of the above and similar relations.

Susceptibility to Warming at Different Periods During Maturation

The foregoing experiments had shown that momentary warming has a favorable action in inciting parthenogenetic development during, but not after, the period of maturation. It remained to determine more precisely the limits of the period of susceptibility to this form of treatment, and the variation in favorability within this period.

For this purpose in each of the series of experiments tabulated in Table IV the eggs of a single female were employed; successive portions of these were warmed to 35° for 70 seconds, beginning about five minutes after removal (at a time when the germinal vesicle had undergone no visible alteration), and thereafter at regular five minute intervals until the separation of the first polar body had taken place. The condition of the eggs at the time of warming was observed in each case in a "control" portion kept under the microscope throughout the entire period of the series. With good eggs from a single female the maturation process progresses with almost uniform velocity in all eggs; the numerous eggs of each portion may thus be considered practically uni-

form so far as regards the stage at which the treatment was applied; all portions were treated exactly alike. The results indicate the existence of a fairly well defined period of maximum susceptibility, lasting for a certain period preceding the completion of the first maturation division. Thereafter conditions become rapidly less favorable.

The results of three satisfactory series of experiments are summarized in the following table:

TABLE IV

The temperature of exposure was 35° and the time 70 seconds in all cases. The condition of the eggs at the time of exposure in each experiment is indicated by the italicized portion enclosed in parentheses. The time itself (interval after removal of eggs from animal) is given in the second column

No.	Time after removal minutes (ca.)	RESULTS		
		Series I July 31, 1907	Series II August 7, 1907	Series III August 12, 1907
1	5	(<i>Germinal vesicle intact and unaltered</i>) Practically all eggs remain immature; no development	(<i>Germinal vesicle intact</i>) Practically all remain immature; no development	(<i>Germinal vesicle intact</i>) Almost all remain immature with intact germinal vesicle
2	10	(<i>Germinal vesicle still unchanged in most</i>) Most eggs remain immature; a few form membranes and develop; a few feeble blastulæ obtained	(<i>Germinal vesicle still intact</i>) Most remain immature; a few mature but none develop	(<i>Vesicle still intact</i>) A fair proportion mature and develop. Some form larvæ, * mostly feeble blastulæ
3	15	(<i>Outline of vesicle becoming indistinct in most eggs</i>) Most form membranes and develop. Many active blastulæ formed	(<i>Outline of vesicle becoming indistinct in a fair proportion</i>) A few eggs mature and cleave. No larvæ obtained	(<i>Germinal vesicle becoming indistinct in a fair proportion</i>) A large proportion mature and develop; a fair proportion form blastulæ and gastrulæ
4	20	(<i>Germinal vesicle is indistinct in nearly all</i>) A larger proportion form larvæ than in Experiment 3 and reach more advanced stage (gastrulæ formed)	(<i>Vesicle disappearing in about one-third of the eggs; rest remain immature</i>) Larger proportion cleavages than in Experiment 3; no larvæ	(<i>About one-half of the eggs are maturing, vesicle indistinct</i>) A fair proportion develop as in Experiment 3

TABLE IV—Continued

No.	Time after removal minutes (ca.)	RESULTS		
		Series I July 31, 1907	Series II August 7, 1907	Series III August 12, 1907
5	25	(Vesicle almost disappeared in nearly all) More favorable than Experiment 4; larger proportion larvæ	(As in Experiment 4; only one-third or so maturing) Larger proportion cleave than in 4; fair number blastulæ	(Germinal vesicle disappearing in ca. one-half of the eggs) Large number good blastulæ and gastrulæ formed
6	30	(Vesicle barely distinguishable in most) Similar to Experiment 5	(About one-third of the eggs maturing) Cleavage as in Experiment 5; no larvæ found	(About one-half of the eggs maturing) Larger proportion of larvæ than in 5
7	35	(Vesicle indistinct in most) Numerous larvæ formed; more favorable than Experiment 6	(Like 6: one-third maturing) Cleavage rather more regular than in 6; fair proportion form blastulæ	(Like 6) More favorable than 6; large proportion of good gastrulæ formed
8	40	(Region of germinal vesicle almost indistinguishable) Larger proportion cleave and more regularly than in 7; next morning most eggs in blastula or early gastrula stage	(One-third mature; first polar spindle visible as clear area at surface of egg) A few blastulæ formed; less favorable than 7	(As before; first polar spindle visible in about one-half of the eggs) Large proportion of eggs form gastrulæ as in 7; rather more favorable
9	45	(Like 8) Most eggs form vigorous gastrulæ by next morning; some reach Bipinnaria stage later. Very favorable culture	(As in 8) Less favorable than 8; no larvæ	(Like 8) Cleavage slower and fewer larvæ formed than in 8; still very favorable
10	50	(Vesicle quite invisible in practically all eggs) Less favorable than 9; till as large proportion form vigorous larvæ a few of which reach early Bipinnaria stage	(Like 8 and 9) Unfavorable; eggs take irregular shapes; no larvæ	(Like 8; polar bodies not yet separated) Less favorable; cleavage more irregular and few form larvæ

TABLE IV—Continued

No.	Time after removal minutes (ca.)	RESULTS		
		Series I July 31, 1907	Series II August 7, 1907	Series III August 12, 1907
11	55	(Like 10) Numerous active larvæ, some reaching early Bipinnaria	(Polar bodies not yet separated) Cleavage irregular; no larvæ formed	(No polar bodies as yet) Cleavage slow and more irregular than in 10; not a single larva
12	60	(Polar bodies not yet separated) Decidedly fewer larvæ and less active than in 11	(Polar bodies beginning to separate) Cleavage irregular; no larvæ	(Polar bodies beginning to separate) Cleavage irregular; no larvæ
13	65	First polar bodies have separated in many eggs) Cleavage much retarded and only a few small irregular blastulæ obtained	(First polar bodies separated in the mature eggs, i. e., one-third of whole) Similar to 12	(Polar bodies separated in maturing eggs) No development
14	17	(Polar bodies separated) Cleavage delayed and irregular; very few larvæ; feeble and abnormal	(Like 13) Like 13; no larvæ	(Like 13) No development

In the above three series a large number of eggs reached the larval stage, Series I being especially favorable. In two other similarly conducted series the eggs proved inferior, only about 10 per cent undergoing maturation. In the first of these series August 10, 1907, larvæ were obtained only from eggs warmed at periods corresponding to Nos. 5 and 8 of the above series; the second, August 13, proved somewhat more favorable, blastulæ resulting from eggs exposed at periods corresponding to Nos. 4, 5, 6, 7, 8 and 9 above with the optimum at Nos. 6 and 7. The suppression of maturation in eggs warmed within 5 to 10 minutes after removal also resulted in both series. Three other similar series—July 29, July 30 and August 3—also showed the same suppression of maturation in eggs heated directly after removal. The eggs in these series were inferior and no larvæ were obtained;

but in the best experiment, that of July 29, the largest proportion of regular cleavages—and in general the most favorable conditions—was found in eggs warmed at stages corresponding to Nos. 8, 9 and 10 of the above table.

In the series tabulated above the following chief uniformities are apparent: (1) Warming shortly after removal (within 5 to 10 minutes, before the germinal vesicle has undergone any apparent change) has the effect of completely preventing maturation; the germinal vesicle remains intact and the egg remains permanently in this condition until disintegration sets in. Such eggs behave in the same manner as do eggs that fail to mature from any other cause—they remain clear and apparently unaltered at a time when mature eggs have undergone complete coagulation and disintegration. (2) Warming at any time after the germinal vesicle membrane has begun to dissolve and before the separation of the first polar body may lead to development and production of larvæ; the proportion of eggs that develop is at first small, but increases rapidly; in general the conditions for development steadily improve until a certain stage is reached—about 40 to 45 minutes after removal at normal summer temperature; at this time the susceptibility of the eggs is greatest and momentary warming is followed by development and the production of active larvæ in a large proportion. Thereafter the susceptibility rapidly declines; and at the time of separation of the first polar body warming results chiefly in abnormal development and larvæ are rarely obtained.

Warming in later periods is still less favorable. In the following two series eggs were treated as above at 10 minute intervals until after the formation of both polar bodies, and after this less frequently until about five hours after removal. Both series proved favorable and showed good agreement; larvæ were most numerous from eggs warmed 10 to 15 minutes before the separation of the first polar body; at the time of separation few or none were obtained, and thereafter conditions became progressively more unfavorable with lapse of time. After both polar bodies had separated the eggs not infrequently failed altogether to cleave or even to produce membranes—a result which agrees with those of the earlier experiments already cited.

TABLE V

Series I, Aug. 18, 1907. Eggs were removed from animal at 11:12 a.m., and warmed to 35° for 70 seconds at following intervals after removal.

Series II, Aug. 20, 1907. Eggs were removed at 9:30 a.m., and warmed to 35° for 70 seconds after the intervals indicated

Interval		RESULT	Interval		RESULT
1	15 m.	(Germinal vesicle beginning to show signs of dissolution) A few eggs reach blastula stage	1	7 m.	(Germinal vesicle unchanged) Practically all eggs remain in immature state; no development
2	25 m.	(Germinal vesicle indistinct in large proportion of eggs) Large number form blastulae and gastrulae	2	17 m.	(A few eggs show beginning maturation) A few feeble blastulae formed
3	35 m.	(Germinal vesicle invisible in most eggs) Good proportion blastulae and gastrulae	3	27 m.	(A small proportion of eggs maturing) Considerable number of blastulae obtained
4	45 m.	(First maturation spindle at surface; no polar bodies separated) Good proportion blastulae and gastrulae	4	37 m.	(Only 5 to 10 per cent eggs maturing) Fair number of blastulae and gastrulae
5	55 m.	(First polar bodies separating) A good many eggs seem to have cleaved regularly but no larvae were obtained	5	47 m.	(First maturation spindle at surface of maturing eggs) More favorable than Experiment 4; good proportion form blastulae and gastrulae
6	1 h. 5 m.	(All maturing eggs have first polar bodies) Mostly irregular cleavages and fragmentation; no larvae	6	57 m.	(Polar bodies not yet separated) A fair proportion of larvae; rather fewer than in Experiment 5
7	1 h. 15 m.	(Second polar bodies not yet separated) After 8 hours most eggs still uncleaved; a few irregular cleavages; no further development	7	1 h. 7 m.	(First polar bodies beginning to separate in maturing eggs) A few larvae; less favorable than 6

TABLE V—Continued

Interval		RESULT	Interval		RESULT
8	1 h. 25 m.	(<i>Second polar bodies separated in large proportion of eggs</i>) Like Experiment 7; after 8 hours mostly uncleaved, or irregular in shape; some are coagulating; no development	8	1 h. 17 m.	(<i>First polar bodies separated in maturing eggs</i>) Cleavage irregular; hardly any reach swimming stage; a single blastula found
9	1 h. 35 m.	(<i>All maturing eggs with two polar bodies</i>) After 8 hours eggs uncleaved; largely without membranes and in process of coagulation; no development	9	1 h. 27 m.	(<i>Second polar bodies not yet formed</i>) After 7 hours egg irregularly cleaved or irregularly shaped and uncleaved; no larvæ formed
10	1 h. 45 m.	(<i>Like 9</i>) After 8 hours uncleaved and irregularly shaped; largely without membranes and coagulating; no development	10	1 h. 37 m.	(<i>Second polar bodies in almost all maturing eggs</i>) After 7 hours markedly different from Experiment 9; most mature eggs have membranes but are uncleaved; a few irregular cleavages or fragmentations; good many coagulating; no development
11	1 h. 58 m.	After 8 hours eggs are irregular and uncleaved; many are without membranes and coagulating	11	1 h. 47 m.	(<i>All maturing eggs with two polar bodies</i>) Similar to 10; mostly with membranes but uncleaved and irregular; some are coagulating
12	3 h. 35 m.	Marked difference from 11; after 6 hours practically all eggs are without membranes and coagulated	12	1 h. 57 m.	(<i>Like 11</i>) After 7 hours eggs are irregular, uncleaved or fragmented; no development
13	4 h. 5 m.	Similar to 12; after 5 hours eggs are without membranes and coagulated	13	2 h. 7 m.	Similar to 12
14	4 h. 35 m.	Similar to Experiments 11 and 12.	14	2 h. 22 m.	After 7 hours eggs uncleaved, irregular or fragmented

TABLE V—Continued

Interval		RESULT	Interval		RESULT
15	5 h.	The same as above; eggs do not form membranes and coagulate within 5 hours	15	4 h. 44 m	After 4 h. 15 m. almost all mature eggs found coarsely coagulated and in process of disintegration, mostly (but not all) without membranes
			16	5 h. 35 m.	After 3½ hours mature eggs are in process of coagulation; only a few have membranes

I find no record in my notes of the condition of the membranes in Experiments 12 to 14 of Series II. In this series membrane-formation is not so completely absent as in Series I in the stages succeeding the completion of maturation; but presumably the proportion of eggs without membranes increased steadily from Experiment 11 to Experiment 16 where only a few were formed.

In both of the above series the eggs show a progressively increasing inability to respond to momentary warming after the maturation process is complete. The proportion of eggs that fail to form membranes also increases with the lapse of time in the post-maturation stages. Cleavage becomes irregular or fails altogether; the curious result also appears in the experiments made some time after the complete separation of the second polar body that the tendency to coagulation, typical of mature unfertilized eggs, is markedly accelerated. This effect is conspicuous in eggs warmed at a stage of four or five hours after removal, as seen in both of the above series; the coagulative process is well advanced in such eggs within three or four hours after warming; while in mature eggs not exposed to this treatment, or warmed at an earlier stage, coagulation does not become evident until some hours later. The process, as Loeb has shown, is oxidative in nature; warming in post-maturation stages has thus the effect of accelerating oxidations leading to a coagulative disintegration of the egg-substance. An earlier experiment performed with another object in view shows the same result: Eggs were removed at 11:30 a.m. August 5, 1907; about

3 h. 45 m. later they were warmed to 35° for the periods indicated.

1	35° 60 s. (3:15 p.m.)	3 h. later (6:15) only a few eggs have membranes; most are unaltered; no cleavage
2	35° 65 s. (3:16 p.m.)	At 6:20 condition similar to Experiment 1; only a few eggs have membranes; these are dead and in process of coagulation
3	35° 70 s. (3:18 p.m.)	At 6:21 the mature eggs are all opaque and coagulated; only a few have membranes; these uniformly show the greatest disintegration
4	35° 75 s. (3:20 p.m.)	At 6:21 condition of eggs similar to Experiment 3
5	35° 80 s. (3:22 p.m.)	Similar to Experiments 3 and 4

In the unfertilized unwarmed control at 6:23 p. m. the eggs remained quite uncoagulated; warming has thus hastened the coagulative change in the mature eggs, and especially in those with membranes, which uniformly showed the most advanced disintegration.

What are the conditions of this varying susceptibility to the above form of treatment at these different periods in the life of the unfertilized egg? One event, occurring shortly after the removal of the egg from the animal to normal oxygen-containing sea-water, seems of fundamental significance, viz: the dissolution of the membrane of the immature egg-nucleus or germinal vesicle. This event naturally must precede the maturation divisions that follow; but quite apart from this it seems to form the condition of a profound change in the properties of the egg-cytoplasm. Delage²¹ has found that enucleate egg-fragments of *Asterias* are insusceptible to fertilization before the germinal vesicle has undergone visible change; but that very soon after its membrane has begun to show indication of dissolution, merogonic fertilization first becomes possible; a little later, when the membrane has become invisible—although the area of the former germinal vesicle may still be seen, often with nucleolus intact—the fragments of cytoplasm are completely and readily fertilizable. These observations demonstrate that the essential feature of maturation, so far as the cytoplasm is concerned, is not the separation of the polar bodies, but simply the removal of the barrier between the nuclear and the cytoplasmic

²¹ Delage: Archives de zoologie expérimentale et générale, Sér. III, T. 9, p. 285, 1901.

areas; they also show that the nuclear membrane acts as a semi-permeable membrane with reference to certain substances contained within it. The critical event, therefore, which conditions this remarkable change in the properties of the cytoplasm is, according to Delage, the passage of certain nuclear constituents (*suc nucléaire*) into the cytoplasmic area (*loc. cit.*, p. 289). These substances he suggests may either change the osmotic pressure of the cytoplasm, or may influence the rate of oxidations, or may be of the nature of particular electrolytes or enzymes. He was unable to produce by artificial means any developmental changes in such egg-fragments.

The precise nature of the change induced in the egg-cytoplasm in consequence of the dissolution of the germinal vesicle is as yet unknown. The fact that the egg, if not fertilized within a few hours, readily undergoes an oxidative change involving a coagulation of the cytoplasmic colloids seems to point to an acceleration of oxidations in that region—due possibly, as lately suggested in an interesting paper by Mathews,²² to the liberation of oxidases formerly confined to the nuclear area; if oxidases are of nuclear origin, as certain facts seem to indicate,²³ such a consequence would naturally follow; the periodic dissolution of the nuclear membrane in mitotic cell-division would thus have the significance of providing for the distribution of the oxidases (synthesized in the nucleus) throughout the cytoplasmic area; this would naturally result in a periodic acceleration of oxidation processes in the cell. Lyon²⁴ has in fact shown that the production of carbon dioxide by the dividing egg follows a rhythm parallel with that of the nuclear division; and Loeb²⁵ has connected these oxidations with the synthesis of nucleins from the compounds of the cell-protoplasm—a process which is likewise characterized by a rhythm parallel with that of the mitotic process.

This general interpretation, though suggested by quite different

²² Mathews: American Journal of Physiology, vol. xviii, p. 94, 1907.

²³ Cf. the references in my paper On the Oxidative Properties of the Cell-nucleus, in American Journal of Physiology, vol. vii, p. 412, 1902.

²⁴ Lyon: American Journal of Physiology, vol. xi, p. 52, 1904.

²⁵ Loeb: Biochemische Zeitschrift, vol. i, p. 183, and vol. ii, p. 34, 1906.

considerations, is in striking agreement with the view propounded by Conklin²⁶ some years ago in his studies of karyokinesis in the *Crepidula* egg. Some of his conclusions on the physiology of this process should be quoted. "The nuclear membrane appears to permit the passage of materials inward but not outward during the resting period; whereas the escape of nuclear material into the cell is brought about by the disappearance of the nuclear membrane during karyokinesis." In *Crepidula* there can be demonstrated cytologically "a very extensive interchange of material between the nucleus and the cytoplasm;" "a large part of that most characteristic nuclear substance, the chromatin, passes into the cytoplasm in the form of oxychromatin during every cell-cycle, while a relatively small part is reserved for the purpose of reproducing the daughter-nuclei." This passage of nuclear material (presumably nucleo-proteid in nature) into the cytoplasm is regarded as a fundamentally important condition of the subsequent changes undergone by the latter. These phenomena appear to be characteristic of mitosis in general and essentially similar conditions have been described for a number of cells. In the starfish egg by far the greater part of the chromatin is set free in the cytoplasm during the first maturation division.²⁷ In *Chætopterus* also the greater part of the germinal vesicle consists of a "residual substance" which is set free in the cytoplasm at the first maturation-division and plays an important part in the future development.²⁸ It is natural, in view of the probable nucleo-proteid nature of at least certain enzymes, to regard the above "oxychromatin" or "residual material" as consisting—at least in part—of the ferments concerned in the chemical processes—largely oxidative in their nature as shown clearly by the conditions in the starfish-egg—that determine the later characteristic changes in the cytoplasm. The ascertained cytological facts are thus in essential harmony with the above hypothesis.

Whether the change in the cytoplasm depends primarily on increased oxidations or on other conditions is scarcely decided as

²⁶ Journal of the Academy of Natural Sciences of Philadelphia, second series, vol. xii, pt. 1, 1902.

²⁷ Wilson and Mathews: Journal of Morphology, vol. 2, p. 334, 1895.

²⁸ Cf. F. R. Lillie: Journal of Experimental Zoology, vol. iii, p. 153, 1906.

yet. My own observations agree with Delage's and those of later observers in indicating that the dissolution of the nuclear membrane is in some way associated with a well-defined alteration in the capacity of the egg for further development. Momentary warming previously to this event not only fails to result later in cleavage, but it has the effect of completely preventing the change in question and with it the entire maturation-process. On the other hand, as already seen, the same treatment applied at any time after the beginning of the maturation-changes (until the separation of the first polar body) may lead to development and the production of larvæ. The properties of the cytoplasm thus must undergo a profound change the nature of which remains to be determined.

One normal sequence of the dissolution of the germinal vesicle is a change in the reaction of the egg-cytoplasm toward membrane forming agencies. Membrane-formation now promptly follows warming, or the entrance of a spermatozoön, or the momentary action of a fatty acid or fat solvent; while in the immature egg this structure is usually not formed under these conditions.²⁹ Yet, although as a rule eggs that remain permanently immature as above do not form fertilization membranes on warming, this is by no means invariably the case. I have recorded numerous instances in which momentary warming has produced perfectly normal membranes in immature unfertilized eggs. In general such eggs belonged to lots that were unfavorable as regards capacity for development, so that the membrane-production may be considered as evidence of a certain abnormality. The following instance will illustrate: in the series of August 2, 1907, cited above, in which eggs were warmed at 5-minute intervals (until the separation of the first polar body in those maturing), the majority failed to mature, and the developing mature eggs in no case reached the free swimming stage. In this series most of the permanently immature eggs, after subjection to the momentary warming process, formed quite typical uniform membranes indistinguishable from those found in fertilized mature eggs; this was especially true of

²⁹ Cf. Loeb: University of California Publications, Physiology, vol. ii, p. 150, 1905.

those warmed at periods of 10 to 15 minutes after removal; after an hour (at which time most mature eggs had formed polar bodies) the proportion of immature eggs that formed membranes had declined considerably, and at later stages only a few were formed. This is not in the least an isolated observation, but is fairly typical of what I have frequently observed; the ability of immature eggs to form membranes seems in general best marked shortly after removal, and diminishes after an hour or more in sea-water. An observation made on the same lot of eggs showed that spermatozoa may also induce membrane-formation in immature eggs; sperm was added at 1 h. 25 m. after removal; the next morning a fair proportion of the mature eggs had formed larvæ; and nearly all of the immature eggs showed perfectly typical sharply defined fertilization-membranes; otherwise these eggs remained unchanged. Spermatozoa are known to enter immature starfish-eggs,³⁰ but typically to produce no membranes. Under certain conditions however, not definitely understood (eggs "over-ripe" or otherwise not quite normal), membranes may be formed as just seen, either by spermatozoa or through an artificial agency. The explanation may be as follows: normally the possibility of membrane-formation depends on the passage of certain substances from the nucleus to the cytoplasm, since the beginning of maturation is a prerequisite; in the above eggs however the permeability of the germinal vesicle membrane is abnormal, so that the substances necessary to the membrane-formation, which ordinarily are unable to traverse the nuclear membrane, are now able to effect this passage and to enter the cytoplasm. The latter then reacts to heat or the entrance of the spermatozoön by forming a membrane in the manner characteristic of mature eggs.

It is interesting also to note that such immature eggs show no other change in their properties; they remain clear and unaltered for prolonged periods and show no greater tendency to disintegrate than do normal immature eggs—a fact apparently contradictory of Loeb's view that the separation of the membrane involves an acceleration of oxidative processes in the egg. In mature

³⁰ Wilson and Mathews: *Journal of Morphology*, x, p. 319, 1895.

eggs, however, there is an obvious difference in the conditions; the *entire* contents of the germinal vesicle—not only those substances that can pass the nuclear membrane—have become mingled with the cytoplasm; and in fact mature eggs differ from immature eggs in undergoing the typical disintegration much more rapidly after forming membranes, as shown above. It is quite possible that for the oxidations concerned in the post-maturative disintegration of the cytoplasm there is needed the presence of specific substances derived from the nucleus—e. g., oxidases, or enzymes or proferments of some other kind, or certain activators—and that these substances merely find better conditions for their activity after the separation of the fertilization-membrane than before. In their absence membrane-formation would in itself effect no essential change in the condition of the cytoplasm. Membrane-formation alone is thus quite ineffective—unless accompanied by certain other and independent changes—in accelerating oxidations in the egg-cytoplasm.

The effect of momentary warming in *preventing* the dissolution of the germinal vesicle is curious and difficult to explain. The process itself, as shown by Loeb³¹ some years ago, depends on oxidations, since it is prevented by acidulation of the sea-water or by depriving the eggs of free oxygen. One of his observations seem analogous to the one under discussion; exposure of unripe egg even temporarily (as for 15 minutes), to acidulated sea-water (100 cc. sea-water + 5 cc. $\frac{N}{10}$ HNO₃) prevented the eggs from maturing after retransfer to normal sea-water. An oxidative process therefore which normally leads to the dissolution of the nuclear membrane within a few minutes after the eggs are laid, if checked before that time is ordinarily not resumed and the eggs remain immature. But why should temporary *warming* at this stage produce a similar result? The expectation would be that by such treatment the oxidations, as well as the other chemical processes in the egg, would be accelerated, and that a process like maturation, dependent on oxidations, would be furthered rather than prevented. Evidently warming, during the brief period that

³¹ Loeb: Archiv für die gesammte Physiologie, xciii, p. 59, 1902.

normally precedes the solution of the nuclear membrane of the immature egg, in some manner inhibits the oxidations on which this change depends. Just why this effect should result remains for the present obscure; possibly several distinct chemical processes are concerned, having different coefficients of acceleration by rise of temperature; at the higher temperature the available oxygen may enter into a quite different reaction from that on which the maturation-change depends; the latter would then be prevented through a deficiency of available oxygen. Oxidations in one set of processes may easily involve reductions in another if the supply of free oxygen is limited. What is remarkable is that maturation is prevented *permanently* by warming at this stage. Warming after the germinal vesicle has broken down has no effect on the course of maturation, the polar bodies forming in the usual manner; and after this process is complete the eggs, as already seen, may proceed to cleave and develop without fertilization. Apparently conditions unfavorable to maturation produce a *permanent* prevention of the process only if they act during the brief period immediately following the deposition of the eggs; this is for some season a critical stage, and if the maturation process is not then begun it fails altogether. In harmony with this interpretation is the well known fact that starfish eggs which show no signs of maturing by twenty minutes or so after removal from the animal to normal sea-water remain immature permanently.

The effects of momentary warming at stages succeeding the dissolution of the germinal vesicle vary, as just shown, according to the exact period at which the treatment is applied. As already seen, membrane-formation and development may result from warming very soon after the vesicle begins to lose its distinct outline. The conditions are at first unfavorable, only a small proportion of eggs forming membranes, and still fewer developing to a free-swimming stage. In general, as indicated by Table IV, the proportion of favorably developing eggs shows a progressive increase until an optimum stage is reached—usually about 15 or 20 minutes before the separation of the first polar body; warming at the time of separation of this polar body rarely results in larvæ, and in later stages the conditions become steadily less favorable with lapse of time.

The conditions of this change of susceptibility are at present unknown. I have endeavored to determine if a similar variability exists in respect to fertilization by spermatozoa; and the result has appeared that although normal fertilization is possible throughout a far greater period in the history of the egg (namely, at any time after maturation has begun until several hours after its completion) a very similar variation in the degree of susceptibility to the fertilizing influence does in fact exist. Conditions for fertilization by spermatozoa are at their best during the maturation period, at or about the time of separation of the first polar body; and later they become less favorable. There is thus a certain parallelism between the conditions of artificial fertilization by momentary warming and of normal fertilization by spermatozoa. The following table gives the results of two series of experiments. Spermatozoa were added to successive portions of eggs, taken in each series from a single female, at the indicated intervals after removal from the animal. The condition of the eggs at the time of fertilization is indicated by the italicized portion in parentheses.

Four other similar series of experiments were performed with, in general, very similar results. In all of these the most favorable time for fertilization was either *before* or *about at the time of* the separation of the first polar body; eggs fertilized at periods of one to three hours after the completion of maturation gave few or no larvæ, and these were mostly abnormal. These experiments agree in indicating that the egg gives the best response to the fertilizing influence of the spermatozoön at or near the time of separation of the first polar body. After the separation of the second polar body the proportion of developing eggs undergoes rapid decline. It is however *possible* for eggs at such stages to give normal larvæ on fertilization, although the optimal conditions are found at earlier stages.

On comparison with the results of momentary warming a certain agreement is seen. The egg responds best to both fertilizing influences at or near the time of separation of the first polar body although rather *before* than after this event in the case of warming. This agreement is of some further interest as indicating that the essential determining conditions of the initiation of the develop-

mental process are similar in normal and in artificial fertilization. Further and more precise analysis of these conditions is needed; in particular, examination should be made of the susceptibility of eggs, at different periods during and after maturation, to the

TABLE VI

Series I. August 24, 1907

1	35 m.	(<i>First polar body not yet separated</i>) Practically all mature eggs form blastulae and gastrulae
2	57 m.	(<i>First polar body about to separate</i>) A large proportion of larvæ; seems less favorable than Experiment 1
3	1 h. 17 m.	(<i>First polar body separated</i>) Favorable; practically all eggs form active larvæ
4	1 h. 50 m.	(<i>Mature eggs have both polar bodies</i>) Somewhat less favorable than Experiment 3; a large proportion of good larvæ
5	3 h. 10 m.	(<i>1 h. 15 m. after separation of 2d polar body</i>) Marked contrast to Experiment 4; most eggs dead and coagulated next morning; only a few larvæ

Series II. September 2, 1907

1	50 m.	(<i>First polar body not yet separated</i>) Practically all mature eggs form larvæ
2	1 h. 30 m.	(<i>First polar body in all maturing eggs</i>) Very uniform and normal looking lot of larvæ; next morning are mostly active; early gastrulae
3	1 h. 55 m.	(<i>Both polar bodies in all maturing eggs</i>) Less favorable than Experiment 2; larvæ less numerous and less well developed; a considerable number small or otherwise abnormal
4	2 h. 55 m.	(<i>More than one hour after separation of 2d polar body</i>) Unfavorable; relatively few larvæ and these mostly abnormal; most eggs dead and coagulated next morning
5	4 h. 30 m.	(<i>Nearly 3 h. after 2d polar bodies</i>) Still less favorable. Most mature eggs are dead and coagulated next morning; a few larvæ, mostly small, thick-walled, or irregular in shape. None normal
6	5 h. 25 m.	A few larvæ; most eggs dead and coagulated next morning

fertilizing influence of momentary warming in dilute potassium cyanide solutions. This method, as will shortly be shown, produces results far superior to those obtained by simple warming in normal sea-water; and it is possible that after the completion of

maturation eggs may be found to respond to some such form of treatment. As yet I have made no investigation of these relations. Probably the most appropriate form of treatment will be found to vary at different stages, according to the physiological condition of the egg. The experiments about to be described indicate that the state of oxidation of the egg-protoplasm is a most important factor; and it seems not unlikely that the above differences in response at different periods may be found to depend largely on varying conditions of oxidation at different stages.

Effects of Combining Momentary Elevation of Temperature with the Action of Cyanide Solutions

The supposition that momentary elevation of temperature produces its effects on the eggs through an acceleration of oxidation processes suggested itself early in the investigation. The beautiful experiments of Loeb³² had shown the importance of the presence of oxygen in the action of hypertonic solutions on the *Strongylocentrotus* egg. I therefore tested the effects of warming starfish eggs under conditions that exclude the influence of accelerated oxidations. For this purpose sea-water containing potassium cyanide to $\frac{M}{20000}$ concentration was employed. In this medium intracellular oxidations are greatly retarded if not almost altogether suppressed, as shown by the fact that mature eggs remain for days without undergoing the typical coagulative disintegration, which, as Loeb has shown, is dependent on oxidations. In the following experiments the eggs were warmed to 35° for 70 seconds while in KCN sea-water, to which they were transferred in some cases directly from normal sea-water, in others from $\frac{M}{20000}$ KCN in which they had been allowed to lie for varying periods of time. After warming, the eggs were transferred in some experiments directly to normal sea-water, in others to $\frac{M}{20000}$ KCN at normal temperatures, whence, after varying intervals, they were transferred to sea-water.

The influence of previous treatment with cyanide solutions on the development of eggs warmed momentarily in normal sea-water

³² Loeb: *Biochemische Zeitschrift*, vol. i, pp. 189, 1906, *et seq.*, and preceding papers in *University of California Publications*.

was first tested. Sea-water containing KCN in $\frac{M}{3000}$ to $\frac{M}{1000}$ concentrations acts in the same manner as sea-water deprived of its dissolved oxygen by a current of hydrogen or otherwise; the maturation process is checked, and may be resumed on retransfer to sea-water if too long an interval has not elapsed. As shown above, after the maturation-process has progressed beyond a certain stage, starfish eggs become less and less susceptible to the influence of momentary warming. It can be shown that the process (whatever its nature) which deprives the egg of this susceptibility is retarded or prevented along with the maturation by the addition of cyanide to the sea-water. This is illustrated by the following experiments:

Eggs were placed August 21, 1907, 20 to 25 minutes after removal from the animal, in sea-water containing $\frac{M}{2000}$ KCN. In this solution they were left for 2 h. 30 m. They were then transferred to normal sea-water (which was changed to free the eggs of adhering cyanide) and portions were warmed to 35° for 70 seconds at successive intervals of ten minutes until the appearance of the first polar body. At the close of the period of exposure to the cyanide solution the eggs were almost all in an early maturation stage with invisible germinal vesicle. Maturation was resumed in normal sea-water; the polar bodies began to separate after an interval of 1 h. 30 m.; a certain delay in the resumption of the process is thus indicated. Eggs were warmed at the following intervals after return from cyanide solution to normal sea-water and the results were as tabulated in the following table:

TABLE VII

1	5 m.	Eggs form membranes and some reach well-advanced cleavage stages. No larvæ formed
2	15 m.	More favorable; a considerable number of larvæ formed
3	25 m.	Considerable number of active larvæ
4	35 m.	Seems rather less favorable than Experiment 3; still a good proportion form larvæ
5	45 m.	Less favorable; only a few larvæ
6	1 h. 5 m.	Unfavorable; no larvæ formed
7	1 h. 35 m.	Unfavorable; no larvæ

An experiment of August 17 showed a similar result: Eggs placed ten minutes after removal in $\frac{M}{2000}$ KCN, left in this solution two hours, then washed for 10 minutes in normal sea-water and warmed, gave a considerable number of larvæ.

In these experiments the eggs were not warmed directly in the cyanide solution; but were first transferred to normal sea-water and then after an interval, subjected to the warming process in the latter medium. The largest proportion of larvæ developed from eggs warmed within 15 minutes to half an hour after this transfer (Experiments 2 to 4); later the conditions became less favorable. The failure to reach advanced stages in Experiments 5 and 6 may seem to contradict the rule found above that optimal conditions for parthenogenesis are found at a time approaching that of the separation of the first polar body. The influence of the cyanide must, however, be taken into account; as will be seen later the presence of cyanide during the warming process improves the conditions greatly, and the greater favorability in the earlier experiments in all probability depends on the relative briefness of the period succeeding removal from the cyanide solution. These eggs were thus exposed at a relatively favorable stage of maturation while still to a certain degree under the influence of the cyanide. Such a combination of circumstances would be favorable to development.

In the experiments now to be described the eggs were exposed to the high temperature while in the cyanide-containing sea-water. In the first series they were placed in $\frac{M}{2000}$ KCN solution at an early maturation stage, and after varying intervals were warmed to 35° for 70 seconds in the same solution and then transferred directly to sea-water. The result has appeared uniformly that under such conditions a far larger proportion of eggs develop, and development is more rapid and more nearly normal, than in eggs warmed in normal sea-water without the cyanide treatment.

The following series will illustrate:

TABLE VIII

August 24, 1907. Eggs were removed at 2:55 p.m. and after 30 minutes in normal sea-water were transferred to $\frac{M}{2000}$ KCN in sea-water. In this solution, after the intervals indicated, successive portions were warmed to 35° for 70 seconds, and immediately transferred to normal sea-water. In each experiment the eggs were allowed to settle and the sea-water was changed and this washing process was repeated a second time

	Period in KCN before warming	RESULT
1	30 m.	Almost all eggs form larvæ, largely more or less irregular blastulæ; some reach early Bipinnaria stage
2	50 m.	Larger proportion of active and normal larvæ than in Experiment 1; practically all mature eggs form larvæ of which many reach the early Bipinnaria stage
3	1 h. 10 m.	Rather less favorable than Experiment 2; many larvæ reach early Bipinnaria
4	1 h. 30 m.	Majority of mature eggs form larvæ a good many of which are small and thick-walled; very active swimmers. A fair proportion reach early Bipinnaria
5	2 h. 35 m.	Sharp contrast to Experiment 4; all eggs die in an early stage. No larvæ formed

Control warmed in normal sea-water: Three portions were warmed in normal sea-water (without previous cyanide treatment) at respectively 30, 40 and 50 minutes after removal. All three formed numerous active larvæ; the conditions, however, were decidedly less favorable than with the cyanide-treated eggs; most eggs died in early stages, development was slower, and the resulting larvæ were less active and normal than in the favorable cyanide cultures.

Sperm-fertilized controls: Sperm was added to five successive portions at 35 m., 57 m., 1 h. 17 m., 1 h. 50 m. and 3 h. 10 m. after removal; numerous active larvæ were obtained in all but the last; on the whole, the best sperm-culture was inferior to the best cyanide-culture and reached less advanced stages of development.

A second series on August 27 gave similar results though the eggs were not so favorable. The result was, however, all the more striking since the best cyanide cultures were found to give a larger proportion of active normal larvæ than were obtained with sperm fertilization, even at the most favorable time.

TABLE IX

August 27. Eggs were removed at 9:45 a.m.; left 30 minutes in normal sea-water; then transferred to $\frac{M}{2000}$ KCN, and after the designated intervals warmed to 35° for 70 seconds in this solution, from which they were transferred directly to sea-water; this was changed twice to remove all traces of cyanide

	Time in KCN solution	RESULT
1	45 m.	All maturing eggs form membranes and cleave to an advanced stage. Only a few form blastulæ; these are relatively feeble and abnormal
2	60 m.	The majority of mature eggs form blastulæ; larvæ are largely abnormal, with walls of unequal thickness; the number of active and normal larvæ is greater than in the sperm-fertilized control
3	1 h. 25 m.	Less favorable than Experiment 2. Eggs mostly stop short in early cleavage stages; only a few larvæ obtained
4	1 h. 45 m.	Still less favorable. Eggs cleave irregularly and very few form blastulæ
5	2 h. 15 m.	Like Experiment 4; a few feeble abnormal blastulæ
6	2 h. 45 m.	Eggs stop short in early cleavage; no larvæ
7	3 h. 50 m.	Like Experiment 6. Cleavage irregular; no larvæ

Control warmed in normal sea-water: Three portions warmed respectively 30, 40 and 50 minutes after removal gave only a few small abnormal blastulæ.

Sperm-fertilized control: Portions were fertilized 30 m., 49 m., 1 h. 11 m., 1 h. 27 m., 1 h. 55 m., 2 h. 55 min., 4 h. 20 m. after removal; in the best cultures (30 m. and 49 m.) only one-third to one-half of the eggs formed blastulæ of which a large proportion were abnormal.

In experiments 3 to 7 many eggs cleave irregularly and stop short in early cleavage stages. A remarkable peculiarity of such eggs is that after 24 hours the blastomeres still remain clear and uncoagulated and apparently living, though undergoing no further cleavage. This condition is in striking contrast to the fate of eggs fertilized either normally or artificially without cyanide treatment and whose development also ceases in early stages; in such eggs the blastomeres rapidly undergo the typical coagulative disintegration characteristic also of mature unfertilized eggs. The cyanide has apparently permanently modified the cell-protoplasm in such a manner as to check or prevent the oxidations on which this breakdown depends.

A third similar series (August 23) should also be mentioned briefly. In this series the control eggs, warmed in normal sea-water without previous cyanide treatment, gave no swimming larvæ; and the sperm-fertilized eggs gave only a few, from a portion fertilized about 40 minutes after removal; these fertilized later (1 h. 10 m., 1 h. 25 m., 1 h. 50 m., 3 h. 15 m. and 5 h. 15 m.) gave no larvæ. The eggs were thus typically "unfavorable." A portion of the unfertilized eggs was placed in $\frac{M}{2000}$ KCN 30 min-

utes after removal, warmed to 35° for 70 seconds after the indicated intervals in the cyanide sea-water, and then transferred as above to fresh sea-water. The results were as follows:

1	25 m. in KCN	Large proportion of vigorous larvæ formed
2	55 m.	More favorable than Experiment 1; after 24 hours numerous blastulæ and gastrulæ were present
3	1 h. 30 m.	Less favorable; relatively few blastulæ were formed and these were mostly abnormal
4	2 h. 50 m.	Unfavorable; very few eggs reach blastula stage

A fourth series (August 29) gave an even more striking result. Eggs were placed, 40 minutes after removal, in $\frac{M}{2000}$ KCN, warmed to 35° for 70 seconds after the following intervals in this solution, then transferred to normal sea-water which was changed as usual. The control eggs warmed in sea-water at 40, 50 and 60 minutes after removal gave only a few blastulæ, the great majority dying and disintegrating at an early stage. In the best of the several sperm-fertilized portions only one-third to one-half of the mature eggs formed blastulæ which were largely feeble or otherwise abnormal. The results were as follows:

1	35 m. in KCN	Next morning the dish was full of vigorous normal-looking blastulæ and early gastrulæ; condition much better than in the best sperm-fertilized control
2	60 m.	Decidedly less favorable than Experiment 1; a good proportion of eggs form larvæ, but these are less active and normal than above
3	1 h. 35 m.	Still less favorable; nevertheless a large proportion have formed larvæ; these are largely irregular in form and somewhat feeble in movement

In each of the above four series of experiments a far larger proportion of eggs produced larvæ after treatment with cyanide for an appropriate length of time than after simple warming unaccompanied by such treatment; and the development was more nearly normal and resulted in the production of larger and more vigorous larvæ. The results were indeed comparable in the best instances to those obtained with normal sperm-fertilization; in fact, in the last two series better conditions were obtained with the artificially fertilized eggs than with those fertilized in the natural manner.

It is noteworthy that a certain time of exposure to the cyanide solution—apparently about one hour or somewhat less—produces optimal conditions for development; after more prolonged exposure warming tends to result in abnormal development; in Tables VIII and IX the proportion of eggs that reach a larval stage is seen steadily to diminish with increase in the time of exposure to the cyanide beyond an hour or so, and the larvæ tend to become thick walled, irregular in shape, or otherwise abnormal. After exposure for more than two hours to the cyanide few eggs develop to a free-swimming stage. This change in the condition of the eggs points to the existence of certain processes other than oxidations which continue unchecked in the presence of cyanide; there are no doubt hydrolyses of various kinds, and it may reasonably be inferred that both kinds of processes are concerned in the changes that render the egg capable of parthenogenetic development. Suppression of oxidations for a time, during which the hydrolyses proceed unchecked, appears then to be favorable to bringing the eggs into a condition in which they respond readily to momentary warming; but if the hydrolyses unaccompanied by oxidations are allowed to proceed too far, lack of coördination in the succeeding developmental processes seems to result, as shown by the increased proportion of unfavorably developing eggs. Normally a certain balance between the oxidative and the hydrolytic processes must exist; possibly a disturbance of this balance may be an important condition in the initiation of the developmental process. Such an interpretation is at least suggested by the foregoing results.

It should be pointed out that simple exposure to cyanide solutions without warming has no influence in initiating development in these eggs—at least under the above conditions. In the second of the two series tabulated above a portion of eggs was transferred from the cyanide solution to sea-water, without warming, at the time of each experiment of the series. None of these eggs formed membranes or showed any other sign of development and all were dead and coagulated next morning. The momentary elevation of temperature is thus essential. Since hydrolytic processes are relatively unaffected by cyanide, we may infer that hydrolyses are accelerated to at least four or five times the original velocity during

the period of warming—probably to an even greater degree, since the above results on membrane-formation (pp. 381, et seq.) indicate a much higher temperature-coefficient of acceleration for such processes under the conditions prevailing in the cell. Indications, then, seem to point to an acceleration of hydrolytic processes, combined with a repression of oxidations, as an important condition in the initiation of development in these eggs. That hydrolyses are in fact accelerated seem to be indicated by the conditions of the membrane-formation; this event occurs quite normally *in* the cyanide solution; it appears to be dependent on a hydrolysis which is greatly accelerated by a rise of temperature; and presumably other hydrolyses in the egg would be similarly affected by the same change of conditions. Membrane-formation seems to afford a clear proof that certain processes, not dependent on oxidations, are markedly accelerated by momentary warming, and that certain critical changes in the developmental capabilities of the egg-protoplasm may result from such momentary acceleration.

Naturally it is impossible for eggs treated as above to develop while remaining in the cyanide solution; the transfer to oxygenated sea-water is indispensable. This transfer however need not be immediate. It is possible to keep eggs, after warming under the above conditions, in cyanide sea-water for a certain not too prolonged period before transfer to sea-water. No visible change occurs during the stay in the cyanide solution, but on transfer to normal sea-water development proceeds normally. Indeed, under certain conditions such after-treatment with cyanide has proved highly favorable to development as the following experiments illustrate:

In these experiments the eggs, after remaining for a certain time in cyanide-containing sea-water, were warmed momentarily as above and brought to normal temperature in that medium; then after an interval they were returned to normal sea-water. A certain stay in the cyanide solution after warming proved in every case decidedly favorable.

The following series will illustrate:

TABLE X

Series I. September 7. Eggs were transferred 30 minutes after removal from the animal, to sea-water containing $\frac{M}{2000}$ KCN; after an interval of ca. 40 minutes they were warmed in this solution to 35° for 70 seconds; thence transferred to cyanide solution at normal temperature; from this, after the designated intervals, portions were transferred to normal sea-water.

Exposure to KCN after warming		RESULT
1	(control) o (to sea-water directly)	Not favorable; comparatively few larvæ formed
2	5 m.	A striking contrast to the control; nearly all mature eggs form active larvæ; the majority of these gastrulate and many reach the early Bipinnaria stage
3	10 m.	Similar to Experiment 2; very favorable; numerous early Bipinnariæ result (with mesenchyme and with the three intestinal divisions plainly marked)
4	20 m.	A very good vigorous lot of gastrulæ were obtained, but rather less favorable than in Experiments 2 and 3; relatively few reach advanced stages

Controls warmed in normal sea-water 35, 45 and 55 minutes after removal gave considerable numbers of good larvæ.

Sperm-fertilized control, fertilized one hour after removal, gave also a large proportion of larvæ, though fewer than in the best experimental cultures; development was also less rapid.

A number of eggs were left in the KCN solution after warming until next morning (23 hours); they were then clear and uncleaved and all had typical membranes. On transfer to normal sea-water none underwent development, and next day all were dead and disintegrated.

In the above series of experiments a marked increase in favorability resulted from the brief after-treatment with cyanide. In those next to be described a greater range of exposure to the cyanide solution was employed; otherwise the procedure was the same.

TABLE XI

September 2, 1907. The eggs were left in sea-water for 45 minutes after removal; then transferred to $\frac{M}{2000}$ KCN for one hour, warmed to 35° for 70 seconds in this solution, retransferred to $\frac{M}{2000}$ KCN at normal temperature, and thence, after the designated intervals, transferred to normal sea-water which was changed twice to remove all cyanide.

Interval between warming and return to normal sea-water		Condition after 24 hours
1	0 (control; directly to sea-water after warming)	Large number of normal well-advanced gastrulæ
2	4 m.	A decidedly larger proportion of swimming larvæ than in Experiment 1; numerous normal gastrulæ are formed
3	14 m.	A large proportion of larvæ; on the whole less uniform and less advanced than in Experiment 2
4	24 m.	Like Experiment 3 but with somewhat larger proportion of abnormalities; still, many good active gastrulæ
5	44 m.	Distinctly less favorable than Experiment 4; fewer larvæ than in Experiments 3 and 4, mostly blastulæ or imperfect gastrulæ
6	64 m.	Similar to Experiment 5; a good proportion of larvæ, largely abnormal; fair number of gastrulæ
7	1 hr. 24 m.	Relatively unfavorable; a smaller proportion of larvæ and these mostly small thick-walled blastulæ; relatively few gastrulæ, which are less advanced than in above experiments
8	2 h. 34 m.	Considerable number of thick-walled blastulæ, but fewer than in Experiment 7. No regular blastulæ and no gastrulæ. Many eggs have stopped short in early cleavage stages
9	23 h.	Development stops in early stages and eggs disintegrate; none reach larval stages

Controls warmed in normal sea-water, 50 and 65 minutes respectively after removal, gave a fair number of blastulæ after 24 hours, of which a few were beginning to gastrulate. As compared with Experiments 1 to 6 above, the larvæ are fewer and in a less advanced stage of development.

Of the sperm-fertilized controls, those warmed within 1 h. 30 m. after removal gave a large number of normal active larvæ.

On examination, after 24 hours, of eggs left in the cyanide solution, all were found with membranes, round, clear, uncoagulated and uncleaved; many, however, showed little pseudopodia-like projections, and frequently small portions of the surface-protoplasm had become detached from the egg. While cleavage is impossible in the KCN solution, there appears nevertheless to have been some slight cytoplasmic activity in these eggs.

In a third series similar conditions were found; in this series the eggs were unfavorable and very few larvæ resulted even in the best sperm-fertilized control. A relatively small proportion of eggs formed larvæ in the best experiments; still, exposure to $\frac{M}{2000}$ KCN

solution for some minutes after warming gave decidedly better results than were obtained from eggs transferred directly to sea-water without after-treatment with cyanide. The eggs were removed from the animal at 10 a.m. September 4, 1907; at 10:35 they were placed in $\frac{2}{1000}$ KCN; and after 55 minutes were warmed to 35° for 70 seconds and then replaced in cyanide solution at normal temperature, whence, after the intervals used, they were transferred to normal sea-water. Here the eggs brought into sea-water directly after warming in cyanide solution gave no larvæ; while eggs after exposed to cyanide for only 5 minutes yielded considerable numbers of good gastrulæ, proving in fact more favorable than the best sperm-fertilized control; 10 minutes after-treatment on the other hand gave few larvæ; and eggs left respectively 20, 35 and 50 minutes in cyanide after warming gave successively fewer and fewer; while none resulted with after-exposures of 1 h. 10 m., 1 h. 30 m., 2 h. 50 m. and 4 h. 20 m.

These experiments indicate clearly that checking of oxidation processes during a certain interval after warming acts favorably under certain conditions; if this interval is prolonged for more than a few minutes conditions become rapidly less favorable, possibly, as suggested above, in consequence of the progress of certain hydrolytic processes unaccompanied by oxidations. The striking increase in the proportion of developing eggs under the treatment used above, and also in the rate and normality of the development, suggests strongly that anaërobic conditions—at least at certain stages—form an important factor in the initiation of development in starfish eggs. Oxygen is necessary to the developmental process itself; but the internal changes that impart to the egg the distinctive power of automatic development seem best induced under conditions that must very effectually prevent most intracellular oxidations—at least those conditioned by the presence of enzymes. The above results indicate therefore that momentary elevation of temperature—assuming that its essential action is the acceleration of chemical processes in the egg-substance—must affect primarily other processes than the oxidative; in brief, acceleration of these processes, presumably hydrolytic in nature, simultaneously with a suppression of oxidations, appears

in some manner to result in changes leading to the initiation of development.

After-treatment with cyanide also acts favorably in the case of eggs that have been warmed in normal sea-water without previous exposure to cyanide solutions. The following experiments will illustrate:

TABLE XII

September 9, 1907. Eggs were removed at 10:15 a.m. and the majority began to mature. After 43 minutes they were warmed in normal sea-water to 35° for 70 seconds. One portion (A) was then transferred to normal sea-water; a second portion (B) to $\frac{M}{2000}$ KCN solution, and from this portion were transferred at the following intervals to sea-water

Interval in KCN solution before transfer to sea-water		RESULT
1	0 (control A; untreated with KCN)	Almost all eggs die in early cleavage stages; only one or two blastulæ found
2	5 m.	Most eggs die, but a distinctly larger proportion reach the blastula stage than in the control and these are better developed
3	10 m.	Similar to Experiment 2; larvæ are decidedly more active, numerous, and well-developed than in the control; some have entered the early gastrula stage after 24 hours
4	25 m.	Conditions are still more favorable; larvæ are more numerous and more typical than in Experiments 2 and 3
5	60 m.	Similar to 4; good many early gastrulæ (mostly more or less abnormal) after 24 hours

A portion of eggs fertilized with spermatozoa about one hour after removal gave a good proportion of larvæ; largely small and thick-walled or otherwise abnormal. The eggs were thus not especially favorable.

The proportion of eggs developing to blastulæ and farther, while not large in the above series, was decidedly increased by the after-treatment with cyanide, and development proved both more rapid and more nearly normal in eggs thus treated. The best conditions were found in Experiments 4 and 5. Too prolonged after-exposure to cyanide affects the egg injuriously, the proportion of abnormal larvæ being greater in Experiment 5 than in Experiment 4.

A repetition of this experiment, with a larger range of exposure to cyanide, gave a similar result (Table XIII).

TABLE XIII

September 10, 1907. Eggs were removed at 10:30 a.m.; the majority underwent maturation. After 45 minutes the eggs were warmed to 35° for 70 seconds as usual; a portion (for control) was placed immediately in normal sea-water; the remainder in $\frac{M}{2000}$ KCN solution, whence, after the intervals indicated, portions were transferred to sea-water

Time in KCN solution		RESULT
1	0 (control)	Nearly all eggs are dead after 24 h., but a few blastulæ and gastrulæ have developed. (A second portion of eggs warmed about 65 m. after removal also gave a few larvæ, mostly irregular blastulæ)
2	6 m.	Most eggs die but larvæ are distinctly more numerous and active than in the control; a fair proportion are gastrulating after 24 hours.
3	11 m.	Similar to Experiment 2; a large proportion of larvæ are gastrulating after 24 hours.
4	21 m.	Rather less favorable than Experiments 2 and 3; a fair number of larvæ formed
5	36 m.	Similar to Experiments 2 and 3; a fair proportion of larvæ are gastrulating after 24 hours
6	59 m.	A good proportion of larvæ after 24 hours, largely well formed early gastrulæ
7	1 h. 30 m.	Unfavorable; no larvæ found
8	4 h.	Unfavorable; no larvæ

The Sperm-fertilized control (sperm added 40 m. after removal) gave a large proportion of gastrulæ largely abnormal—irregularly shaped, thick-walled, or sluggish. Very few have gastrulated by 24 hours.

Here also a decided increase in favorability followed after-exposure to the cyanide solution for a not too prolonged period. The results however were less favorable than in the experiments where eggs were exposed to cyanide for some time previously to warming and were warmed in the solution. We may infer that while suppression of oxidations for a certain period after warming is favorable to development in eggs which have previously been well exposed to oxygen, this treatment differs from the preceding in certain very essential particulars, the nature of which requires further analysis. Treatment with cyanide previously as well as subsequently to the momentary warming is essential if the most favorable conditions are to be attained.

On reviewing the general outcome of the experiments described in this section we are led, first, to the conclusion that the *entire* series of events leading to the initiation of development in these eggs includes the changes preceding and following the warming

process, as well as those immediately induced by the latter. Secondly, all of these changes appear to proceed best under conditions of lack of oxygen—in other words, to be essentially anaërobic in their nature. A predominance of anaërobic processes in the changes initiating development implies that an important part is played here by reductions (in the chemical sense), since anaërobic metabolism is always accompanied by the production of strongly reducing substances. The possible part played by such reductions in the processes of cell-division and growth has been discussed by Mathews in the paper already cited; and the above general result is therefore consistent with his view that the production of asters (regarding this phenomenon as an essential feature of mitosis) is the expression of localized reducing processes. I can however hardly see my way clear to the conclusion that the momentary elevation of temperature under anaërobic conditions acts essentially by accelerating reductions and thus producing astral areas. While this is a possible interpretation, it can, as almost purely speculative, serve no particular purpose at present until confirmed or disproved by experiment. Moreover, in the sea-urchin egg the conditions seem of quite an opposite nature. Still, so far as regards the main chemical conditions of the parthenogenetic initiation of development in the starfish egg, the above results appear to indicate very definitely a subordination of oxidative processes to those of some other nature.

This conclusion, while opposed to that reached by Loeb in the case of the sea-urchin egg, is in harmony with the recent experimental results of Delage³³ with the starfish. In this form parthenogenetic development through the action of carbon dioxide was found to be best obtained in the absence of oxygen; a high concentration of oxygen in the carbon-dioxide-containing sea-water proved definitely unfavorable; and, in general, the lower the proportion of oxygen present, the better were the results obtained. Thus the initiation of development through this means, as well as through momentary warming, appears dependent on processes of an essentially anaërobic nature. Precisely contrary relations

³³ Delage: *Comptes rendus*, vol. 145, p. 218, 1907.

were found by Delage in the case of *Strongylocentrotus*, as had already been determined by Loeb; here the presence of oxygen in the hypertonic solutions is favorable to development. We have thus a striking contrast between the two forms in respect to the part played by oxygen in the initiatory process. This contrast cannot be explained at present; it can only be referred to deep-seated constitutional differences between the two eggs. One further consideration is suggested and should be emphasized here: it must be recognized clearly that the physiological conditions underlying the *initiation* of development—i. e., the bringing of the egg into a condition in which it becomes capable of automatically passing through its characteristic ontogenetic cycle—may be of quite different nature from those on which the developmental process itself depends. This is seen in the fact that notwithstanding the contrast in the conditions of the initiatory process, both the above eggs require the presence of free oxygen for their development. Unexplained constitutional differences between species play a part here, and we are not yet in a position for broad generalization. Nothing but further exact investigation of the conditions of artificial parthenogenesis in eggs of different groups can be expected to bring to light the fundamental conditions common to the different types. For the solution of this problem a systematically inductive procedure seems safest at present.

SUMMARY

1 Momentary exposure of the eggs of *Asterias forbesii*, during the early maturation period, to temperatures of 35° to 38° results in the formation of typical fertilization membranes, followed by the development of many eggs to a free swimming larval stage.

2 The favorable duration of exposure to the above temperatures is very brief, with a well-defined optimum for each temperature; this optimum is approximately 70 seconds for 35° , 40 to 50 seconds for 36° , 30 seconds for 37° , and 20 seconds for 38° . A very rapid rate of decrease in time of exposure with rise in temperature is thus indicated, a rise of three degrees above 35° apparently

trippling the velocity of the process or combination of processes on which the initiation of development depends. The process of membrane-formation shows a similarly high temperature-coefficient of acceleration.

3 The responsiveness of eggs to this treatment varies greatly at different periods in the life of the egg. Warming within five minutes after the removal of the eggs from the animal is ineffective, and has the effect of preventing permanently the dissolution of the germinal vesicle. Warming at any time between the beginning of nuclear dissolution and the separation of the first polar body may result in development and the production of larvæ; the most favorable period is some little time (10 to 20 minutes) before the separation of the first polar body. Warming subsequently to this event tends to produce abnormal form changes or irregular cleavage; after maturation is complete the effect is mainly to accelerate the coagulative change characteristic of mature unfertilized eggs in presence of oxygen.

4 Maturing eggs placed in $\frac{1}{2000}$ KCN solution retain for several hours their susceptibility to development by the above means. A stay of a certain duration in cyanide solution followed by momentary warming in this solution and transfer to sea-water is followed by a striking increase in the proportion of favorably developing eggs. Further exposure of eggs to cyanide solution for a certain period after warming effects a still further improvement in the conditions of parthenogenetic development. Eggs thus treated with cyanide approximate closely, in the rate, character, and favorability of their development, to normally fertilized eggs.

5 Since the essential action of the above dilute cyanide solutions is to prevent intracellular oxidations, the inference is drawn that anaërobic processes play an important part in the series of changes leading to the initiation of development in starfish eggs. Suppression of oxidative combined with acceleration of hydrolytic and reducing processes is indicated as a condition of the initiatory process in these eggs.

THE SEX RATIO AND COCOONING HABITS OF AN ARANEAD AND THE GENESIS OF SEX RATIOS¹

BY

THOS. H. MONTGOMERY, Jr.

WITH TWO FIGURES

This communication presents a study of the numerical proportions of the sexes in *Latrodectus* determined for 41,749 newly hatched young, with briefer observations on such proportions in other spiders; then an account of the general cocooning habits; next an attempt to show that different species of organisms probably have different sex ratios, with an explanation of the origin of such differences.

I *LATRODECTUS MACTANS* FABR.

This is the largest North American Theridiid and it was selected partly because of the ease with which it may be kept, but more particularly on account of the great degree of sexual dimorphism: with the adult males and females so different in form and size it was anticipated that the sexes might be distinguished at the time of hatching, and this hope was realized.

At Austin, Texas, where I have been observing these spiders, the web of this species is found usually on the ground beneath a stone or log, sometimes several feet up within a crevice of a rock wall. The female remains at the upper portion of the web, and uses a niche or cranny as a retreat. Her web is composed of unusually powerful threads, capable of holding the strongest beetles and even of sustaining small stones; indeed I allowed my captives to fasten down with it the glass plates serving as covers for their cages, and this they did so firmly that the glass would not fall when the cages were inverted. Adult males I have found

¹ Contributions from the Zoölogical Laboratory of the University of Texas. No. 89.

only on the webs of females and only from December to February. Here, accordingly, the beginning of the reproductive season is in the early portion of the year. But on the Colorado river about sixty miles northwest of Austin I collected adult males in August. It would seem then that different groups of individuals show different mating periods.

Methods

It was my object to determine not only the general sex ratio of this species, but also the ratio for each successive cocoon of a given spider. Therefore it was necessary to keep females through a whole reproductive season. That the captivity of the mothers did not produce abnormal results will be shown later. Only by controlling individuals in this way can one obtain accurate notes of the times of making and hatching of each cocoon, and also prevent the cocoons from being parasitized. Early in March and April of this year (1907) I collected a number of females and these I have kept until the autumn, up to the close of the time of oviposition. To each of them was devoted a separate cage of pasteboard, most of these cages about three inches high, and most of them triangular with each side about three inches long. A photographic glass plate was used as a cover, and another as a base. All these cages were kept together in a portion of my study where no direct sunlight reached them, and upon the cover of each was laid a paper card that excluded most of the light entering from above; by lifting this card one could look into the web without injuring it or disturbing the inmate. The bodies of their victims, when they have sucked them dry, the spiders drop out of the web; fortnightly, accordingly, I pulled out the bottom glass plates so as to remove these accumulations. Cocoons were also taken out from below, by removing the same plates. Thus the upper portion of the snare where the spider awaits her prey and where she devours it, was never disturbed, and to spare this web as much as possible, food was admitted through a small hole in one side of the cage, this hole being otherwise closed by a cork.

Only living food is accepted, and for this I used large house flies caught in the usual wire traps; sometimes the diet was varied

by larger insects. The spiders were given equal amounts of food, and from the beginning of the experiment until August 8 all were richly fed, and daily except in the colder portion of the spring when food was hard to obtain, so that each of them averaged probably five or six blue bottle flies a day, quite the equivalent of the amount in a state of nature. Between August 8 and 29 they received only two meals, for I was absent; and in September they received only three good meals. Up through the first week in August, which marked the close of reproduction with most of them, these spiders were kept under natural conditions of light, temperature and amount of food. The healthy and active condition of the captives until the middle of August, and the large number of cocoons they produced, evidenced the favorable circumstances under which they were maintained.

Each spider received a separate number, and each cocoon the number of the mother together with the cocoon letter; thus the first cocoon of spider 2000 was 2000A, the second, 2000B, and so on. No cocoon was removed from a cage until several hours after its construction, for when just made the danger of injury to the eggs is greatest; each was lifted out as gently as possible with a pair of forceps, placed in a bottle covered with perforated paper and kept there until the young emerged; these were preserved in 80 per cent alcohol within twenty-four hours of hatching. It is necessary to kill the spiderlings before their first postnatal moult, else they commence to attack each other. This isolation of the cocoons is the only method for preventing the young from dispersing and so becoming lost at the time of hatching.

Three series of females were kept: (1) twelve individuals whose young were allowed to hatch for the computation of the sex ratio; (2) five individuals whose eggs were preserved twenty-four hours after oviposition to test possible voluminal differences; and (3) two individuals kept to test parthenogenesis. More than this number I could not keep well fed. No deaths occurred until August 29, and the nine deaths from then on were probably due to insufficient feeding commencing with the second week of August.

Cocooning Habits

The mating I have not observed, but it probably takes place about the beginning of the year when the adult males are found upon the webs of the females. Wild cocoons are to be discovered as early as February. The cocooning season extends, at Austin, from that month continuously into August. I conclude that it usually terminates in August, for only eight cocoons were made by my spiders after the eighth of that month; of these two were made in September and one in October. Further the last cocoons of a series, especially such dating from the middle of August, are frequently infertile; compare on Table I the last cocoons of 2013, 2016, 2021 and 2031. Females live on after the cocooning season provided they are well nourished.

The completed cocoon is not quite globular but somewhat pyriform, the upper portion having a short stalk to attach it to the object that overarches the web; when fresh it is snow white, when older, yellowish, and its outer coat is markedly resistant and firm. The process of cocooning and oviposition has much resemblance to that of *Theridium*² but *Latrodectus* is less specialized in that she applies the thread mainly by direct application of the spinnerets and rarely by manipulation of the fourth leg pair. The case as seen in the making of cocoon 2020E was as follows: At 5:37 p.m. the mother was seen working at the base, a disc of flossy silk then only 2 mm. in diameter; she hung below it, holding its edges with her three posterior pairs of legs while with her first pair she suspended herself from the web; she was then making 52 applications of her spinnerets per minute. The base was completed at 6:04, an inverted cup with a diameter equal to that of the finished cocoon. Oviposition, with rapidly repeated uplifts of the abdomen against the concave surface of the base, lasted from 6:04 to 6:16. The construction of the cover of the cocoon occupied from 6:16 to 8:19; for the first ten minutes the fourth pair of legs were used to comb out the thread before each application of the spinnerets to the cocoon, after that time these legs were no

² Compare Montgomery: The oviposition, cocooning and hatching of an Araneid, *Theridium tepidarium* C. Koch. Biol. Bull., xii, 1906.

more employed to handle the issuing thread. The rapidity of the applications of the spinnerets was found to be as follows:

from 6:16 to 6:25, 78 applications per minute,
from 6:25 to 6:45, 120 applications per minute,
from 6:45 to 7:30, 125 applications per minute,
from 7:30 to 8:19, 140 applications per minute.

At 8:19 she ceased suddenly, perhaps from exhaustion, then spun again at the rate of 108 applications per minute from 8:28 to 8:32. The cocoon was then completed, and the final touches were to anchor it firmly in the web after cementing it to the roof. Now each time the spider applies her spinnerets to the cocoon she draws out a thread having a length of 5 mm. (the length of the fourth tibia); multiplying the distance of such a thread by the number of applications of the spinnerets, the astounding fact is reached that in spinning the cover alone of the cocoon the spider employs a thread having a total length of about eighty meters. The muscular energy employed is very great, being a rapidly repeated uplifting of the heavy abdomen. Another spider worked on the cover of a cocoon for one hour and fifty minutes, and two others for five hours each.

Oviposition usually takes place in the morning before 6:30 o'clock, and a little later than that one usually finds the process of cover making. In 143 cases oviposition was between midnight and 7 a.m., in eleven cases between 7 a.m. and noon, in eight cases between noon and 6 p.m., and in only one case between 6 p.m. and midnight.

The young make their own way out of the cocoon, usually through a single circular aperture that they make probably by biting; they emerge in rapid succession, and unlike the adults are positively phototropic. In eight cases the hour of emergence was between midnight and 6 a.m., in twenty-eight cases between 6 a.m. and noon, in sixty cases between noon and 6 p.m., and in thirty cases between 6 p.m. and midnight. The afternoon at its hottest hours, between 3 and 5 of the clock, is the most frequent time of hatching. The young do not commence cannibalism until after their first postnatal moult, and the time of this varies

with the temperature as well as with the individual spiderling. Most of those cocoons laid from the middle of June on hatched in nineteen or twenty days, and two in as short a time as seventeen days; longer intervals are characteristic of eggs laid earlier in the year, and the earliest cocoon always takes the longest time to hatch; this is readily seen on comparing successive cocoons in Table I, and shows that the rate of development depends directly upon the temperature.

The total number of cocoons raised by those seventeen spiders that furnished series of them was 187, an average of eleven to each spider. One individual formed eight cocoons, one formed nine, four formed ten each, four formed eleven each, five formed twelve each, while two formed thirteen each, the range thus extending from eight to thirteen. The time interval between successive cocoons varies with the month, so probably with the amount of nourishment, it being shortest in July and August; such intervals may be easily computed from the data given in Table I.

Sexual Dimorphism and the Sex Ratio

On the North American continent there are two good species of the genus *Latrodectus* Walck., *L. mactans* Fabr. and *L. geometricus* Keys., as I have convinced myself by a study of the material in the United States National Museum; for the opportunity of examining this collection my thanks are due to the courtesy of Mr. Nathan Banks. This collection contains specimens of *mactans* from California (San Bernardino, Tulare county, Clemente Island), Texas, New Mexico, Nebraska, District of Columbia, Colorado, Georgia, North Carolina and Oregon; while Marx³ states that it occurs also in Pennsylvania, Ohio and Utah. Though *mactans* shows this wide distribution and is everywhere of rather confined local occurrence it does not appear to have split into geographical races.

The sexes of *mactans* show a marked dimorphism both in size and color, as seen in the following comparison.

³ Catalogue of the described Araneæ of temperate North America. Proc. U. S. Nat. Mus., xii, 1890.

Adult female. Maximum dimensions: abdomen from anterior convexity to spinnerets, 12 mm.; first leg, 21 mm., second leg, 15 mm.; third leg, 13 mm.; fourth leg, 20 mm. Rufous black is the color of the cephalothorax, sternum and legs, and only the metatarsi and tarsi are lighter. The abdomen is deep black, enormous, nearly globular and arched on all surfaces except the ventral; it is marked only by a broad red mark on the venter behind the epigynum, a short red median band just dorsal to the spinnerets, and (rarely) traces of other red spots along the dorsum; at the dorso-anterior border are one or two narrow transverse red marks. In a few specimens from more northern localities the dorsal red spots were prominent, and in one only a pair of oblique red bands on each side. The female is thus shining black with a few red markings.

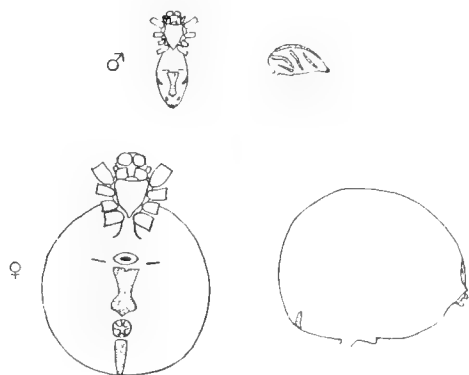


FIG. 1

Adult male. As shown by the accompanying figure the male is much smaller than the female, with elongate abdomen; in this figure the abdomina are shown from ventral and lateral views and the stippled areas denote the light markings; he has also proportionately longer and more slender legs. The cephalothorax and sternum are both pale brown with darker borders, the cephalothorax also with a darker median stripe. The legs are yellow; the distal ends of the femora and patellæ are darker, and there are two rings of the same dark color at the distal ends of the tibiæ. The abdomen in seven specimens has a broad white dorso-median

band extending from near the anterior end to the spinnerets; at the antero-dorsal boundary a transverse white band that extends down on both sides; on each side behind the latter are two oblique white bands; all these bands are narrowly edged with black. On the venter there is a broad white mark which is on each side bordered by black, and on each side of the spinnerets are three oblong black spots. The remainder of the abdomen is pale brownish flecked with white. In one male there was a red line inclosed within the dorso-median white band of the abdomen, and deep black filling all the spaces between the abdominal white bands.⁴

The males in the instar just preceding the adult stage have the abdomen larger and more rounded, the legs proportionately thicker, and the abdomen colored like the young female, namely, dull or deep black, with a medio-dorsal white band including a red one, an arched transverse white band anteriorly, two oblique narrow white bands on the sides, and a broad white band on the venter.

The newly hatched of both sexes are yellow with black stripes on the abdomen as follows: two narrow parallel stripes along the dorsum, two broader ones on the venter, and three (often broken) oblique stripes on each side. Thus the color of the adult male retains the color pattern of the young much more than does the adult female, for the latter becomes to great extent deep black. In color, size and activities the male is decidedly more embryonic.

So far all observers, with the exception of Doumerc, have held the newly hatched of spiders to be sexually indistinguishable. At that period the genital plates are quite simple, and the pedipalpal tarsus of the male is not different from that of the female. On sectioning the spiderlings of *L. mactans* I could not distinguish

⁴I have described *mactans* rather more fully than might seem necessary for our present purposes, but this is called for on account of the present confusion with regard to the American species. For this reason I will give briefly the characters in which *geometricus* differs from *mactans*. The male of *geometricus* differs only in having two pairs of black spots just behind the middle of the dorsum. The female of *geometricus* differs from the female of *mactans* in having the dimensions of all parts of the body slightly smaller but the abdomen much smaller, the legs pale colored with dark rings; in *geometricus* also the general color of the abdomen is pale brown, more rarely black, and always marked by lighter markings on the dorsum and sides. Further, the cocoon of *geometricus* has the surface beset with numerous slender, cylindrical villi, each from 1 mm. to 1.5 mm. in height, while the cocoon of *mactans* is quite smooth. *L. geometricus* occurs in California and Jamaica.

ovaries from testes, for each is simply a small paired chord of germ cells of an early generation. But careful comparisons demonstrate that there are two constant forms of the newly hatched spiderlings, with the following differences.

1 Individuals which have the abdomen wider and deeper, with the dorsum much more strongly arched and the pedicel placed further back. In such individuals the abdomen is almost always distinctly larger. These are females.

2 Individuals which have the abdomen narrower and less deep, with the dorsum only moderately arched or not infrequently flattened or even indented. Such individuals have in almost all cases the abdomen smaller. These are males.

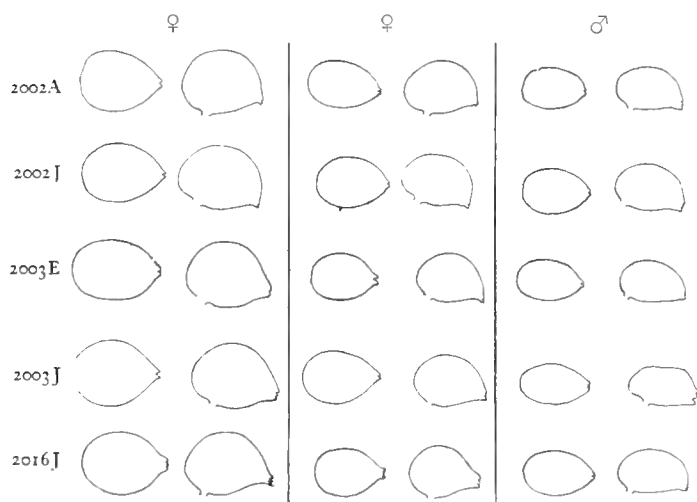


FIG. 2

Intermediates do not occur between these two groups. In Fig. 2 I have illustrated such differences by showing in outline the abdomina of spiderlings from several cocoons. To the left is the number of the particular cocoon, and on a line with it outlines of the abdomina of the largest and smallest females, and of the largest male of that cocoon, each abdomen shown on lateral and ventral view. It is hardly necessary to add that plane drawings cannot

represent these differences as clearly as the rounded originals do. Now these are like the form and size differences of the abdomina of the adults; the adult female has the more arched abdomen with the pedicel placed further back, while the male has the narrower and flatter abdomen with the pedicel situated further forward. Therefore I conclude, and no reasonable objection can be entertained to this opinion, that those spiderlings most resembling in these particulars the adult females are females, and those most similar to the adult males are males. And it will be recalled that intermediate individuals are not found, and one can separate rapidly and unhesitatingly the spiderlings from a given cocoon into two lots.

I had hoped to be able to distinguish the sexes at a still earlier stage of growth, by constant differences in egg sizes, and for this purpose preserved the eggs (all at the age of about twenty-four hours) of the cocoon series of five different spiders. But this expectation was not realized. The eggs in any cocoon differ somewhat in volume, not greatly, but these size differences form a graduated series and not two unbridgable groups. The sexual differences of the hatched spiderlings being constant only with regard to form but not always with regard to size of the abdomen explains why we do not find female eggs always larger and so distinguishable from male eggs.

The following table represents the proportions of the sexes in those cocoons of which the young were preserved shortly after hatching. The first column gives the number of the mother and the letters designating her successive cocoons. The second gives the day of oviposition, and the third the time interval between oviposition and hatching. The succeeding columns state the number of unhatched eggs, of males, of females, and the male ratio: under the male ratio I understand the quotient obtained by dividing the number of males by the number of females.

TABLE I

Cocoon	Oviposition	Time to hatching	Unhatched eggs	♂	♀	♂ r. tio
2000		days				
A	April 11	41	18	333	39	8.5
B	May 5	32	19	228	57	4.
C	25	25	11	270	75	3.6
D	June 5	20	0	147	121	1.2
E	12	20	71	247	47	5.2
F	22	19	10	140	95	1.4
G	July 1	19	5	132	165	.8
H	8	19	11	146	102	1.4
I	16	19	42	145	94	1.5
J	25	19	136	92	69	1.3
K	Aug. 4	18	63	60	76	.7
2002						
A	April 14	48	193	7	71	.09
B	May 3	31	0	280	27	10.3
C	21	25	0	243	33	7.3
D	31	22	16	208	53	3.9
E	June 9	19	9	186	68	2.7
F	15	20	1	261	25	10.4
G	23	19	9	292	21	13.9
H	July 1	20	4	325	20	16.2
I	8	20	3	242	18	13.4
J	20	19	1	176	15	11.7
2003						
B				404	2	202.
C	April 15	40	2	300	46	6.5
D	May 8	33	0	301	9	33.4
E	23	25	0	355	20	17.7
F	June 3	21	0	313	37	8.4
G	10	20	0	319	18	17.7
H	17	21	0	331	12	27.5
I	25	20	0	316	17	18.5
J	July 2	20	0	493	18	27.4
2008						
A				239	6	39.8
B	April 15	40	222	13	47	.2
C	May 6	33	0	256	38	6.7
D	20	25	0	246	25	9.8
E	29	22	0	309	22	14.

TABLE I—Continued

Cocoon	Oviposition	Time to hatching	Unhatched eggs	♂	♀	♂ ratio
		<i>days</i>				
2008						
F	June 7	19	3	367	31	11.8
G	12	19	0	355	27	13.1
H	18	20	0	250	50	5.
I	25	19	0	307	28	10.9
J	July 1	19	2	287	40	7.1
K	7	19	60	237	15	15.8
2012						
A			8	526	93	5.6
2013						
A				646	6	107.6
B	April 11	41	33	577	48	12.
C	May 7	32	3	316	15	21.
D	24	26	1	430	9	47.7
E	June 4	21	1	404	20	20.2
F	12	20	0	398	10	39.8
G	23	20	1	432	12	36.
H	30	20	3	319	16	19.9
I	July 16	20	3	432	12	36
J	26	20	2	282	9	31.3
K	Aug. 5					
2014						
A				525	1	525.
B	April 15	38	30	284	34	8.3
C	May 13	29	1	366	19	19.2
D	28	23	0	474	24	19.7
E	June 8	20	2	448	40	11.2
F	17	20	1	393	10	39.3
G	26	20	5	395	24	16.4
H	July 2	20	6	432	22	19.6
I	12	19	2	367	9	40.7
J	24	19	3	328	16	20.5
2015						
A			0	519	27	19.2
B	May 6	32	0	419	15	27.9
C	23	25	0	507	21	24.2
D	June 3	20	0	441	22	20.
E	12	20	0	440	4	110.
F	22	20	0	405	18	22.5

TABLE I—Continued

Cocoon	Oviposition	Time to hatching	Unhatched eggs	♂	♀	♂ ratio
2015		<i>days</i>				
G	July 1	20	1	486	17	28.5
H	8	20	1	399	8	49.8
I	19	19	0	348	14	24.8
J	29	18	0	316	5	63.2
K	Aug. 6	18	1	276	14	19.7
2016						
D	April 11	40	1	286	33	8.6
E	25	37	3	278	29	9.5
F	May 14	29	31	207	49	4.2
G	25	25	5	280	45	6.2
H	June 5	23	18	172	59	2.9
I	12	20	10	193	21	9.1
J	21	20	6	191	21	9.
K	28	21	121	59	42	1.4
L	July 6	20	76	105	35	3.
M	13	28	143	10	0	10.
N	30					
O	Aug. 27					
2017						
A	May 7	32	2	208	32	6.5
2020						
B				399	16	24.9
C	April 15	40	23	533	71	7.5
D	May 19					
E	28	25	4	311	37	8.4
F	June 10	20	4	287	73	3.9
G	19	21	0	311	40	7.7
H	28	20	5	204	50	4.
I	July 6	20	21	214	94	2.2
J	18	19	3	282	89	3.1
K	27	19	1	210	72	2.9
L	Aug. 7	17	3	42	72	.58
2021						
B	April 11	41	1	643	25	25.6
C	29	36	3	387	18	21.5
D	May 19	26	0	434	45	9.6
E	June 13	20	4	412	58	7.1
F	12	19	3	429	18	23.8

TABLE I—Continued

Cocoon	Oviposition	Time to hatching	Unhatched eggs	♂	♀	♂ ratio
2021		days				
G	June 22	19	4	391	24	16.2
H	30	19	3	331	33	10.
I	July 9	19	1	373	16	23.3
J	18	19	0	392	10	39.2
K	30	17	3	194	56	3.4
L	Aug. 6	18	37	260	45	5.7
M	Sept. 25					
2022						
A				588	6	98.
B	May 6	32	4	247	10	24.7
C	24	25	1	346	31	11.1
D	June 3	20	4	293	28	1.4
E	10	20	15	245	51	4.8
F	17	20	6	269	32	8.4
G	25	19	4	297	32	9.2
H	July 2	20	3	382	29	13.1
I	10	18	5	256	24	10.6
J	19	19	7	231	44	5.2
K	27	19	8	248	26	9.5
L	Aug. 8	21	62	66	52	1.2
2023						
A	April 27	38	42	118	96	1.2
2030						
A				410	7	58.5
B	May 13	31	0	199	42	4.7
2031						
A				14	61	.2
B	April 16	37	6	294	47	6.2
C	May 11	30	52	172	60	2.8
D	25	27	301	11	28	.39
E	June 4	21	279	49	50	.98
F	11					
G	18	23	228	15	9	1.6
H	26	21	256	44	33	1.3
I	July 2					
J	11					
K	18					
L	28					
M	Aug. 24					

Notes to Table I

Where a blank is left in the column headed "oviposition" it indicates that the cocoon was found when the mother was captured, the day of oviposition therefore unknown; where a blank is left in the column "Time to hatching" it signifies either that this was not determined, or else that the eggs did not hatch; where a blank occurs in the column "unhatched eggs" it signifies that these were not counted, while a blank in the remaining columns indicates that the eggs did not hatch. Certain cocoons need further explanation, as follows:

2002K. Made September 18, too late to be entered into this table; it did not hatch.

2003A. Proportion of the sexes not given because many escaped at hatching.

2012A. A wild cocoon the mother of which was not secured.

2013K. Eggs killed by mold, the only such accident.

016A,B. Cocoons hatched when found, perhaps made the preceding season.

2016C. Found April 6 and hatched April 17, but the bottle of young dried up so that they could not be counted.

2017. Other cocoons of this spider were used for the egg series.

2020A. A wild cocoon that proved to be parasitized.

2021A. A wild cocoon found empty.

2023, 2030. Other cocoons of these spiders were used for the egg series.

In the succeeding table these data are so summarized as to bring out the reproductive differences of the several spiders entered in the previous table, the "totals" of the third and seventh columns being averages.

TABLE II

Spider	No. of cocoons that hatched	Average no. of unhatched eggs to a cocoon	Unhatched eggs	♂	♀	Average ♂ ratio
2000	11	35.	386	1,940	940	2.
2002	10	23.6	236	2,220	351	6.3
2003	9	.22	2	3,132	179	17.
2008	11	26.	287	2,866	329	8.7
2012	1	8.	8	526	93	5.6
2013	10	4.7	47	4,236	157	26.9
2014	10	5.	50	4,012	19	20.1
2015	11	.27	3	4,556	165	27.5
2016	10	44.4	444	1,681	334	5.
2017	1	2.	2	208	32	6.5
2020	10	6.4	64	2,793	614	4.5
2021	11	5.3	59	4,246	348	12.2
2022	12	9.9	119	3,468	365	9.5
2023	1	42.	42	118	96	1.2
2030	2	0	0	609	49	12.4
2031	7	160.3	1122	599	288	2.
Totals	127	22.6	2871	37,210	4539	8.19

From these data we infer the following conclusions:

1 The average male ratio (number of the males divided by the number of the females) is 8.19, determined from a count of 41,749 newly hatched spiderlings. Among the progeny of a particular female this ratio was never lower than 1.2 nor higher than 27.5. To a cocoon the average number of hatched males is 292.9, of hatched females, 35.7, and of unhatched eggs, 22.6; the average number of eggs to a cocoon is 351.2. Of the total of 127 cocoons entered in this computation, only 8 showed a male ratio of less than one, and from only one (2016M) did only males emerge and no females, this being the only "unisexual" cocoon. The highest male ratio in any cocoon, excluding the case of 2016M just cited was 202 (in 2003B); in eighteen cocoons the male ratio was 30 or higher.

2 The objection might be raised that the above average male ratio of 8.19 might not be the normal one for the species, but might be induced by the life of captivity of the mothers. Therefore I have considered separately the ratio in cocoons made in the natural state and brought into my study to hatch out. Such cocoons are the following of Table I: 2008A, 2012A, 2013A, 2014A, 2015A, 2020B, 2022A, 2030A, 2031A. These present a total of 3866 males and 223 females, giving the average male ratio of 17.3, considerably higher than the ratio 8.19 obtained from the total of cocoons I raised. Whether this difference is due to difference in the mode of life of the mothers, or rather so the fact (to be brought out later) that the male ratio tends to be highest in the first cocoon of a series, I cannot say. These figures would show at least that the high male ratio of captive cocoons cannot be ascribed to artificial conditions, and indeed there is no reason for thinking that the imprisonment of the mothers could affect this ratio.

3 It will be noticed that the male ratio was determined for each cocoon, accordingly also for the average of all cocoons, from the spiderlings that hatched out because I could not distinguish the sexes before the time of hatching. That is, the male ratio of those eggs that did not hatch could not be ascertained, and this is the single disturbing error in the above calculations. Table I furnishes the number of unhatched eggs for each cocoon, and

Table II the average number for each series, for the 127 cocoons from which young emerged; 2871 eggs were infertile in the 127 cocoons from which hatched 41,749 spiderlings. Were those undeveloped eggs all males, the male ratio would be increased to 8.8; were they all females, decreased to 5.1; yet there is no probability of either of these extreme cases. For when the male ratio is unusually high (30 or higher) the number of unhatched eggs to a cocoon is small, and where the male ratio is unusually small, a good case of which is the series 2031 of Table I, the number of unhatched eggs is generally but not always very high. Therefore it is probable that a large proportion of such undeveloped eggs are males, and consequently the error introduced by such eggs is probably a small one.

Next, as to the cause of lack of development of certain eggs. Mechanical jarring of the freshly laid eggs of spiders has been proved to be fatal to them ever since the observations of Herold, so that the handling of the cocoons in the removal from the cage to the hatching bottle may have prevented the development of some eggs. Yet I believe the arrest of development was rarely so induced, for I took great pains to handle all cocoons with extreme gentleness and, as we see from the third column of Table II, the average number of unhatched eggs to a cocoon varies with the different mothers which would not be the case were it due to accident. Probably, therefore, infertility of eggs is due to lack of fertilization; and indeed the frequent happening of the last cocoons of a series proving most infertile is to be ascribed to the supply of spermatozoa becoming exhausted. To test whether normal parthenogenesis occurs I raised two immature females to maturity without benefit of males; one moulted in March and the other in April, which brought them to the mature condition with fully formed epigyna, and both were allowed to live, with good feeding, until August 30. One of them laid no eggs at all; the other made a cocoon containing a few infertile eggs on June 18, and on June 26 dropped on the floor of the cage, without constructing a cocoon, a mass of eggs that also proved infertile. These two individuals had not been impregnated, and all the eggs laid by them were

infertile, which renders unlikely the occurrence of parthenogenesis in this species.⁵

4 When we compare the male ratios of individual cocoons given in the most right hand column of Table I no constant relation is found between this ratio and the position of a particular cocoon within a series. The ratio may or may not be markedly different in immediately successive cocoons as well as in cocoons at the extremes of the particular series. The male ratio does not regularly increase from the first to the last cocoon, nor does it regularly decrease. Yet it will be noticed that of the twelve larger series of cocoons of Table I, in seven of them the first made cocoon of the series shows the highest male ratio (series 2000, 2003, 2008, 2013, 2014, 2020, 2022).

2 OBSERVATIONS ON OTHER ARANEADS

The only observer who has furnished detailed data upon the sexual relations in spiders is Doumerc.⁶ In the autumn of 1839 he captured a mature female of *Theridion triangulifer* and liberated it in a room where it formed a web in a window frame. On April 23 following it made a first cocoon from which only males emerged and on May 10 a second cocoon from which hatched only males. On June 16 she was impregnated by a male, on June 26 made a third cocoon from which only females emerged and on June 28 a fourth cocoon from which came out only males. Doumerc does not state how he distinguished the sexes of these spiderlings; and for such "unisexualiparous" species he concludes that there are two uteri, one for males and the other for females, and that oviposition does not take place from both at the same time.

In collections adult females are usually more numerous than adult males, due simply to the fact that males in their mature condition are usually short-lived. But during the mating season males are more abundant in most species, certainly among the

⁵ Compare Montgomery: On parthenogenesis in spiders. Biol. Bull., 1907.

⁶ Notice sur les Cocons a pontes unisexuellipares de l'Aranéide *Theridion triangulifer*, Walck. Ann. Soc. Entom., France, 9, 1840.

Argiopids, Theridiids and Lycosids. In genera like Filistata, where the males are rare and where normal parthenogenesis seems to occur, it may be that the male ratio is smaller than 1.

For *Theridium tepidariorum* C. Koch I found⁷ a size difference or dimegaly of the eggs, and concluded it was probable that females emerge from the larger eggs and males from the smaller ones; I showed also that some cocoons contained only large eggs and others only small ones, that therefore some may furnish only females and others only males, and that usually one kind of egg greatly predominates in number. Unfortunately this species is not found at Austin so that I could not keep females to test this point, and in my collection I have only a few bottles of newly hatched young. These specimens exhibit, however, two kinds of individuals that do not intergrade; ones with larger and more arched abdomina, others with smaller and flatter abdomina; since these correspond respectively with the differences of the adult females and males, I take them to be females and males, respectively. Using this criterion I found the proportion of the sexes of the young from four cocoons to be as follows:

TABLE III

Cocoon	Unhatched eggs	♂	♀	♂ Ratio
1058	numerous	3	112	.03
1059	1	431	0	431.
1060	0	186	2	93.
1061	9	237	5	47.4

These examples are too few to allow any conclusions beyond the one that in a particular cocoon one sex greatly predominates. These relations are quite similar to those found by Doumerc for *T. triangulifer*, a particular cocoon having a predominance of a particular sex, but quite different from the relations in *Latrodectus*.

Therefore there is probably not a common male ratio for all species of spiders.

⁷ Probable dimorphism of the eggs of an Aranead. Biol. Bull. xii, 1907.

3 THE ORIGIN AND FIXATION OF SEX RATIOS

There are obviously two sides to the question of sex determination: the one, the process that regulates kind and succession of the sexes of one offspring-unit (totality of offspring of one parent), the other, the process of differentiation of the sexes and the origin of sex ratios. The second question is essentially phylogenetic, but if it should be proved that there is a distinct inheritance of sex then this phylogenetic aspect will come to have an important bearing on the other. We are now concerned with the question of the origin of sex and of sex ratios.

The opinion is fairly general that in most animal species, those exhibiting parthenogenesis excluded, there is no great disparity in number between the sexes. This has followed from a consideration of the numbers of the sexes in man, for so far in no other animal except the horse, is there available any computation of the sexes based upon a count of large numbers of individuals at birth. Only such computations have value, it is hardly necessary to add, that are founded upon the count of the sexes at the time of birth or earlier because the mortality after birth frequently varies with the sex, the males in certain lower animals being more short-lived and less resistant. We have found that in *Latrodectus* the male ratio is 8.19, there being born more than eight males to every female. For man, where the statistics are the only ones more numerous than those of *Latrodectus*, the proportion of males to females in 10,864,950 births is 1036 to 1000, the male ratio therefore 1.03, as taken from the compilation presented by Pike in his Table III.⁸ According to Morgan⁹ for "still-born infants, fully formed, but not alive," Quetelet found 133.5 males to 100 females, a male ratio of 1.33, and Bodio (from whose statistics I compute an average) a male ratio of 1.31. Darwin¹⁰ tabulates the births of English race horses, 25,560 in all, giving 99.7 males to 100 females. For *Bufo lentiginosus* King¹¹ found in individuals that had com-

⁸ Pike: A critical and statistical study of the determination of sex, particularly in human offspring. *Amer. Nat.* 41, 1907.

⁹ *Experimental Zoölogy*. New York, 1907.

¹⁰ *The descent of man*, new ed., New York, 1886.

¹¹ Food as a factor in the determination of sex in Amphibians. *Biol. Bull.*, 1907.

pleted their metamorphosis 241 males to 259 females, a male ratio of slightly less than 1; and other students have constated a low male ratio in Amphibia. These examples, based on cases where relatively large numbers of individuals were counted before the age of maturity, and all with the exception of *Bufo* at or before the time of birth, are sufficient to indicate that different species have different sex ratios, and that the sex ratio may be a quality of the species.

Now when there is a male ratio of 8.19 as in *Latrodectus*, such a proportion of the sexes can be explained neither upon Newcomb's theory of chance¹² nor yet upon Castle's idea of the Mendelian inheritance of sex.¹³ Some other explanation is called for and, as I shall proceed to argue, it is probably to be sought in the factor of selection coupled with segregation.

In the first place the distinction of the sexes is a difference of reproductive power. The female is the reproductive individual, while the male is not reproductive but impregnatory, for females can reproduce without males, as in cases of parthenogenesis, but males are unable to reproduce of themselves. The male is distinctly the less important organism for the perpetuation of the race. There is no reasonable proof for the formula of Geddes and Thompson¹⁴ that the male is more katabolic and the female more anabolic, for that is merely an unfounded statement. The sexual difference is one of degree of reproductive ability.

Probably in the earliest racial species all individuals reproduced in equal measure; this is probable simply because sexual dimorphism implies a rather advanced differentiation, therefore one that should have developed more or less gradually. The origin of sex is most easily conceived in the following manner. Individual variation within a species affects so far as we know every quality, therefore reproductive ability would be a quality subject to variation. Among the fluctuants of a species in which sex had not yet become pronounced there would be a series of individuals

¹² A statistical inquiry into the probability of causes of sex in human offspring. Carnegie Inst. Publ. 11, 1904.

¹³ Castle: The heredity of sex. Bull. Mus. Comp. Zool. Harvard, 1903.

¹⁴ The evolution of sex. London, 1897.

extending from such with the greatest to such with the least reproductive ability; the former would be incipient females, the latter, incipient males. With this difference in reproductive ability would certainly be associated metabolic differences, indeed the latter would probably occasion the former.

Even racially older than observable sex difference is the process of conjugation, which at the start had no immediate connection with reproduction. Conjugation has been fixed by selection in that it aids the race by strengthening the reproductive individuals and making them more efficient in generation, conjugation being in certain Protozoa a form of nourishment.

Conjugation being then of use to the race by strengthening or stimulating the reproductive individuals, selection would preserve those segregations of individuals in which variation with regard to reproductive ability is most marked, for in such species conjugation would be most effective by occasioning the most diverse substance intermixture. Selection coupled with segregation would in time tend to eliminate the means, as the least useful in conjugation, and to preserve those individuals most dissimilar in reproductive capacity. This would terminate in a group of reproductive individuals, females, and of fertilizing individuals, males, not connected by intermediates.

With regard to the sex ratio of a particular species, Pike (*l.c.*) concludes that it may "be looked upon as one of the physiological adaptations of the species, determined by the conditions of its existence. * * * If sex is hereditary, we might reasonably expect that the relative numbers of male and female births in any species would be those which, after deducting the early deaths, would confer upon the species at the period of sexual maturity of its individuals the greatest advantage in the struggle for existence so far as the production of young is concerned." This explanation is to my mind entirely just, and the factors would be, to carry the idea out further than Pike did, those of selection and segregation. Those species continue that survive in the struggle for life, and this struggle is the endeavor to insure offspring. Selection operates by removing those species whose reproductive ability cannot successfully meet this struggle. Therefore selection would

preserve, *ceteris paribus*, those races in which the females are either most reproductive or else most caretaking of their young, and in which there is at the same time a sufficiency of males to insure the needed fertilization of the eggs. Within a given species the male ratio would be subject to individual variation: some females would produce a preponderance of males, others of females. In the breeding area of such a species different groups of individuals would come to show different male ratios, just according to the productive peculiarities of their females, and in agreement with what we understand of the action of segregation or physiological selection in general. There would be groups with an unnecessarily large male ratio, others with the male ratio injuriously small, others with the male ratio just rightly proportioned to the number of females to be impregnated. An excessively high male ratio would be a waste of males, and too low a male ratio a waste of eggs because then all the eggs could not become fertilized; in both these cases there would be an overplus of individuals that would not be of service in procreation. Accordingly, selection would preserve such groups of individuals in which the male ratio is most nicely proportioned, most closely proportioned, to the number of females needing to be impregnated. It would preserve them because they would leave the most offspring. The other segregations of individuals would become eliminated because they include a waste of energies and individuals. Selection and segregation would certainly be efficient factors, while it is more doubtful whether heredity would also play a part.

What the male ratio would be in a particular species would vary with different conditions, and particularly with differences in the mode of life of the sexes. Where the sexes are most alike in general habits of life, where internal impregnation of the female is necessary and where the male cannot impregnate more than one female, the proportion of the sexes would be most equal. Where the males are physically quite as strong or even stronger than the females, and where the male has the habit of impregnating several females, it might be that the male ratio would sink below 1; whether polygamous gregarious species should be reckoned here we cannot say offhand, for the number of males born should be higher

than the number that mate, seeing that many may be killed by direct competition. More generally the male lives a simpler life than the female, is less active both physically and psychically, less fit for the struggle for existence, such as is the male in the spiders we have been considering; in such cases many males die before reaching maturity, and for such species the male ratio would be high. Then where eggs do not require fertilization, as in parthenogenetic generations, selection would remove the males.

Thus the average male ratio of a particular species would be fixed primarily by selection and segregation: these factors would confine in rather narrow bounds the ratio of that particular species. They would keep the number of males rightly proportioned to the number of ova that are to be fertilized, without unnecessary waste of either.

And since the factor of chance and the factor of Mendelian inheritance cannot explain certain specific sex ratios, it is at least suggested that these factors may also fail to determine sex within the offspring unit.¹⁵

¹⁵ It may have some statistical value to append a count of 8796 adult individuals of the Rose chafer, *Macrodactylus subspinosus*, that I made during June, 1901, on individuals collected from one small garden near West Chester, Pa., and which gives a male ratio of 1.31:

Date	♂	♀
June 20	824	638
21	642	683
24	1568	1135
25	678	474
27	623	432
28	654	445
Totals	4989	3807

THE CHROMOSOMES IN *DIABROTICA VITTATA*, *DIABROTICA SOROR* AND *DIABROTICA 12-PUNCTATA*

A CONTRIBUTION TO THE LITERATURE ON HETEROCHROMO-
SOMES AND SEX DETERMINATION

BY

N. M. STEVENS

WITH THREE PLATES

In Publication No. 36 of the Carnegie Institution of Washington, the spermatogenesis of a number of Coleoptera was described, and discussed with reference to the determination of sex. The study of the Diabroticas was begun at Cold Spring Harbor in the summer of 1906, and I wish to express my gratitude to Dr. C. B. Davenport for the privileges granted me both at the Carnegie Institution for Experimental Evolution and in the research laboratory of the Brooklyn Institute. I am also much indebted to Miss Isabel McCracken of Stanford University for material of *Diabrotica soror*, prepared with the greatest care and sent to me in December, 1906, and March, 1907.

The same methods were used as in previous work on the germ cells of the Coleoptera. The germ glands were fixed in Gilson's mercurio-nitric fluid, in Flemming's strong chromo-aceto-osmic solution, and in Hermann's platino-aceto-osmic fluid. Sections 5μ thick were stained with iron hæmatoxylin or with thionin. The aceto-carmin method was used for long series of *Diabrotica soror* and *Diabrotica 12-punctata*.

DIABROTICA VITTATA

In the majority of the Coleoptera previously studied (85.7 per cent), an unequal pair of heterochromosomes was found. The Diabroticas have an odd or unpaired heterochromosome, resem-

bling in this respect the Lampyridæ and Elateridæ as well as many of the Orthoptera and Hemiptera.

In the spermatogonial equatorial plate of *Diabrotica vittata*, we find 21 chromosomes (Pl. I, Fig. 1) of various sizes and shapes. If x be considered the heterochromosome, the others can be mated, forming ten equal pairs. In sections stained with iron hæmatoxylin the division of the testis into several definite regions is very striking. The resting spermatogonia hold little of the stain, while the chromatin of the spermatocytes in synizesis and synapsis stages is very black, and again the spireme stage is pale. The synizesis stage here, as in several other Coleoptera (Stevens '06), appears to be a prolonged telophase of the last spermatogonial mitosis. Fig. 2 shows the appearance of the short, crowded chromatin loops in synizesis. Following this stage comes a period in which the chromosomes are uniting in synapsis, and one finds many nuclei similar to Fig. 3, some of the loops still short as in Fig. 2, others longer and showing a sharp angle or a knob at the point of union of two chromosomes. There is no such definite bouquet stage as in many forms, but one next finds a stage in which there are irregularly disposed loops with many free ends and some sharp angles like those in Fig. 3 (Fig. 4). In this stage the heterochromosome (x) is for the first time evident, condensed against the nuclear membrane. This stage rapidly goes over into the spireme stage (Fig. 5), where all of the chromosomes except the heterochromosome (x) seem to be united into a single spireme thread, and the points of union are no longer visible. The spireme is very pale and the heterochromosome therefore very conspicuous. There is nothing unusual in the prophases of the first division. The spireme segments and splits longitudinally, the daughter elements separate as in Fig. 6, then unite again and form rods, dumb-bells, V's and rings (Fig. 7). The chromosomes in the spindle (Fig. 8) are so attached to the spindle fibers that in metaphase they separate into their univalent components, and go to the poles as short thick V's which mass together but soon separate for the second division without any definite rest stage. The unpaired heterochromosome (x) is of course connected with only one pole of the spindle and does not divide in this division. Fig.

9 is the equatorial plate with the heterochromosome (x) at a different level from the other chromosomes. Equatorial plates of the second division are shown in Figs. 10 and 11, the heterochromosome (x) appearing in Fig. 10, and not in Fig. 11. All of the chromosomes divide in this division giving equal numbers of spermatids and spermatozoa containing ten and eleven chromosomes, respectively. The spermatids (Figs. 12 and 13) contain a chromatin nucleolus (n), which is certainly not the heterochromosome, since it is found in all of the spermatids. As the head of the spermatozoön becomes more and more condensed, the nucleolus gradually decreases in size and finally disappears (Figs. 14 and 15). The ripe spermatozoön has a very long slender head (Fig. 16) which stains intensely black in contrast with the earlier gray stages (Figs. 14 and 15).

DIABROTICA SOROR AND DIABROTICA 12-PUNCTATA

Diabrotica 12-punctata of the eastern United States and *Diabrotica soror* of the Pacific coast states resemble each other so closely that one might easily be mistaken for the other. Both are greenish yellow or yellowish green with twelve black spots on the elytra. Kellogg describes *Diabrotica soror* as yellowish green and *Diabrotica 12-punctata* as greenish yellow. The color varies considerably with the age of the beetle. *Diabrotica 12-punctata* averages larger, shades more on the yellow, and the under side of the abdomen is green or yellow while in *Diabrotica soror* it is black. The color of the abdomen seems to be the one external character by which the two species can always be distinguished; for the size, ground color, and size and fusion of spots are extremely variable in both species.

A small amount of material of *Diabrotica 12-punctata* was collected at Bryn Mawr, Pa., in October, 1906. On examining the sections, it appeared either that the species was polymorphic as to its germ cells, or that there must be two or more sub-species or varieties, and possibly hybrids. It was too late to obtain more material of this kind, so, through the kindness of Miss McCracken, a supply of *Diabrotica soror* was secured for comparison with

the eastern species; and in the summer and autumn of 1907, 100 males of each species were studied by means of aceto-carmin preparations. The character of the chromosomes in the male germ-cells of the two species is precisely the same. About 50 per cent of the individuals examined have nine equal pairs of chromosomes and an unpaired heterochromosome, while the remaining 50 per cent have one, two, three or four additional small heterochromosomes.

DIABROTICA SOROR

Type I

The stages in the spermatogenesis of the first type are in most respects similar to those of *Diabrotica vittata*. The spermatogonial metaphase has nineteen chromosomes (Fig. 17), the unpaired chromosome (x) being the largest. The synizesis and synapsis stages are similar to those of *Diabrotica vittata*, but less conspicuous in sections and the stages are less clear. The changes that occur between the telophase of the last spermatogonial mitosis and the pale spireme stage (Fig. 18) probably take place much more rapidly in this species. A polar view of the metaphase of the first spermatocyte division is shown in Fig. 19, a lateral view in Fig. 20, and a late anaphase in Fig. 21. The odd chromosome is usually found at or near one pole of the spindle in the metaphase (Fig. 20). The bivalents are similar to those of *Diabrotica vittata*, and the first division separates their univalent components. In preparations from Hermann material the chromosomes of the daughter plates (Figs. 22 and 23) often begin to show a vesicular condition and in telophase the heterochromosome (x) forms a vesicle by itself, while the other nine chromosomes are blended together (Fig. 24). Fig. 25 is a later stage taken from a cyst in which some second spermatocyte spindles were present, while Fig. 24 was from a cyst containing a few first spermatocyte spindles. Half of the nuclei in these cysts of course contain no heterochromosome. The rest stage between the two divisions is more pronounced than in *Diabrotica vittata* where the chromosomes are simply massed together in telophase,

and separate for the second division without the formation of a nuclear membrane. The second spermatocyte equatorial plates are shown in Figs. 26 and 27, the heterochromosome (x) appearing in Fig. 26. All of the chromosomes divide in this division, giving, as usual, two equal classes of dimorphic spermatozoa. The spermatids and spermatozoa are similar to those of *Diabrotica vittata*. The chromatin nucleolus is found in the earlier stages but is not visible in stages corresponding to Figs. 14 and 15, and the head of the mature spermatozoön is only about one-half as long.

Type IIa

About two-thirds (33 out of 100 males collected at Mountain View, Cal.) of the individuals belonging to the second type have one additional small chromosome, making twenty in the spermatogonia (Pl. II, Fig. 28). The additional chromosome appears as a second heterochromosome in the growth stages (Fig. 29, s). In the first spermatocyte spindle the larger heterochromosome (x) is found, as usual, near one pole of the spindle, while the smaller one (s) may be in the equatorial plate (Fig. 30) or on either side of it (Figs. 31, 32, 33), closely associated with x or as widely separated from it as possible (Figs. 33 and 31). Fig. 34 is a polar view with the two heterochromosomes near one pole of the spindle. The small chromosome may or may not divide in the first division. In some individuals it almost always (possibly always) divides as in Fig. 35 later than the other chromosomes. In other cases it may be found undivided between the daughter plates (Fig. 36), outside of one of them (Fig. 37), or it may be concealed in the general polar mass of chromatin. In the telophase and brief rest stage (Figs. 38 and 39) it is often quite distinct from the remainder of the chromatin. Whether it divides in this mitosis or goes undivided to one pole or the other seems to be a matter of chance, depending perhaps on the part of the spindle which it happens to enter in the prophase. It seems to be much less automatic in its behavior than the other chromosomes. This peculiarly erratic behavior of the small heterochromosome in the

first division gives, or may give, in the same individual six different kinds of second spermatocytes with reference to this chromosome (s), while there are, as usual, two kinds with reference to the large heterochromosome (x). If the small chromosome goes undivided to the same pole with the odd chromosome (x) (Fig. 33), we have second spermatocytes containing nine and eleven chromosomes (Figs. 40 and 41); if it goes undivided to the other pole (Figs. 31 and 37), the resulting second spermatocytes each contain ten chromosomes, one showing the large the other the small heterochromosome (Figs. 42 and 43); while if it divides, the second spermatocytes contain ten and eleven chromosomes (Figs. 44 and 45). As might be expected one finds two conditions in the second spindle. Either a small daughter chromosome is found outside of the equatorial plate (Fig. 46), or the small chromosome which has not divided in the first division, divides in the second (Fig. 47). Both conditions may be found in the same cyst. It is, of course, in only a few favorable spindles that it is possible to see the small chromosome actually dividing, but the metaphases are readily separated into two classes, one where all of the chromosomes are in the equatorial plate (Fig. 48) and another in which one small chromosome, which from its form and size is evidently a daughter chromosome from the first division, appears outside of the plate and often quite near one pole (Fig. 46). It is therefore quite certain that the small heterochromosome divides in either the first or second division but not in both. Clear daughter plates of the second division have never been found.

The conditions described above lead to the production of two equal classes of spermatozoa with reference to the large heterochromosome (x) and four classes, which may be quite unequal, with reference to the two heterochromosomes.

$$\text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + s \end{array} \right\} \text{variable numbers.} \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \end{array} \right\} \text{variable numbers.} \end{array} \right.$$

If s always went to the same pole with x in the first division the classes of spermatozoa would be as follows:

$$\text{Equal numbers } \left\{ \begin{array}{l} \text{I} \quad 9 \\ \text{II} \quad 9 + x + s \end{array} \right.$$

If s always went to the opposite pole from x , we should get the following results:

$$\text{Equal numbers } \left\{ \begin{array}{l} \text{I.} \quad 9 + s \\ \text{II.} \quad 9 + x \end{array} \right.$$

If s always divided in the first spermatocyte division, there would be four equal classes of spermatozoa:

$$\text{Equal numbers } \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + s \end{array} \right\} \text{equal numbers.} \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \end{array} \right\} \text{equal numbers.} \end{array} \right.$$

A study of seventy or more individuals of this kind gives the impression that the small heterochromosome most often divides very late in the first division, but it is certain that there is considerable individual difference. In some cases nearly every anaphase of the first division shows s dividing; in others, it is rarely or never seen dividing in the first spindle, and as stated above, all of the various possibilities have been found in one individual.

Type IIb

Fifteen out of the same 100 males of *Diabrotica soror* had two small heterochromosomes in addition to the eighteen ordinary chromosomes and the large heterochromosome x . These are shown in a spermatogonial plate (Fig. 49). The three heterochromosomes may also be seen in a growth stage (Fig. 50), a prophase of the first division (Fig. 51), lateral and polar views of the metaphase (Figs. 52 and 53) and an anaphase (Fig. 54). Fig. 55 is an equatorial plate of the second division. When two small heterochromosomes are present both may go to either

pole of the first division spindle, one to each pole, or one or both may divide as in Fig. 54. The resulting combinations in the spermatozoa are as follows:

$$\begin{array}{l} \text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + s \\ 9 + 2s \end{array} \right. \left. \begin{array}{l} \\ \\ \end{array} \right\} \text{variable numbers.} \\ \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + s \\ 9 + x + 2s \end{array} \right. \left. \begin{array}{l} \\ \\ \end{array} \right\} \text{variable numbers.} \end{array} \right. \end{array}$$

Type IIc

Three out of the same 100 specimens had three small heterochromosomes, as shown in Fig. 56, a growth stage, 57 and 58, metaphases of the first division.

Type II d

One individual had four such small heterochromosomes which may be seen in Figs. 59-65, growth stages and first spermatocytes. Here one may find all of the possibilities with respect to division and distribution of the small chromosomes. The possible combinations in the spermatozoa are therefore as follows:

$$\begin{array}{l} \text{Equal numbers} \left\{ \begin{array}{l} \text{I} \left\{ \begin{array}{l} 9 \\ 9 + 1s \\ 9 + 2s \\ 9 + 3s \\ 9 + 4s \end{array} \right. \left. \begin{array}{l} \\ \\ \\ \end{array} \right\} \text{variable numbers.} \\ \\ \text{II} \left\{ \begin{array}{l} 9 + x \\ 9 + x + 1s \\ 9 + x + 2s \\ 9 + x + 3s \\ 9 + x + 4s \end{array} \right. \left. \begin{array}{l} \\ \\ \\ \end{array} \right\} \text{variable numbers.} \end{array} \right. \end{array}$$

There were no spermatogonial plates of type IIc and IId which could be counted, and in no case, though many ovaries have been fixed and sectioned and others examined with aceto-carmine, has it been possible to determine the number and character of the chromosomes in the female.

DIABROTICA 12-PUNCTATA

Exactly the same conditions as to the small heterochromosomes prevail in *Diabrotica 12-punctata* collected at Bryn Mawr, Pa., as in *Diabrotica soror* at Mountain View, Cal. Out of the first 100 males examined in October, 1907, 51 had no small heterochromosome, 35 had one, 11 had two, 2 had three and 1 had four, while in *Diabrotica soror* the numbers for the five corresponding classes were 48, 33, 15, 3, 1.

A few figures only will be given for *Diabrotica 12-punctata*. As in many other Coleoptera, spermatogonial equatorial plates in which the chromosomes are well enough separated for accurate counting are rarely found. The one shown for *Diabrotica soror*, type IIb, in Fig. 49, was drawn from an aceto-carmine preparation in which the chromosomes had been separated by pressure on the cover-glass. Figs. 66 and 67 are spermatogonial plates of *Diabrotica 12-punctata*, type I and type IIa, drawn from sections. There is some overlapping here, but no doubt as to the number in either plate. Growth stages for the five classes are shown in Figs. 68, 69, 70, 71 and 72. The larger size of both nucleus and chromosomes in Fig. 72 is due to its having been drawn with the same power from an aceto-carmine preparation. These figures also serve to show something of the diversity of form of the odd chromosome (x). When no small heterochromosome is present it usually is nearly spherical (Figs. 18 and 68). Where one or more of the small chromosomes are found, it is as a rule somewhat elongated (Figs. 69 and 70), often irregular in form (Fig. 72), or much elongated and bent in U-form. Whether this difference indicates some influence exerted by the presence of the smaller heterochromosomes, or marks the individuals containing the small chromosome as a separate species is not at present clear.

In both *Diabrotica soror* and *Diabrotica 12-punctata* the small heterochromosomes are usually quite closely associated with the larger one (x) in the growth stages, but this is by no means invariably true. It is not at all unusual to find them separated in some cells and in one individual it was noted that the two were more often widely separated. (Figures to illustrate this have been thrown out for lack of space.)

Fig. 73 shows a metaphase of the first spermatocyte from the one individual of this species which had four small chromosomes. Figs. 74 and 75 are anaphases from the same section, from an individual with two small chromosomes, showing in one case (Fig. 74, s_1 and s_2) both dividing, in the other (Fig. 75) one dividing (s_2) and the other (s_1) passing undivided to the same pole with the odd chromosome (x). In general, these small chromosomes are remarkably uniform in size. One case, however, was found among the aceto-carmin preparations where an unusually small one was constant for the individual (Figs 76-78). This very small chromosome was not found dividing in the first spermatocyte and it could not be followed in the second division. In one cyst the spireme was segmenting, later than usual, into dumb-bell shaped bivalents (Fig. 77), as in *Tenebrio molitor* (Stevens '05, Pl. 6, Figs. 177-179).

As in the other species of *Diabrotica* it has not been possible to find favorable stages for counting the chromosomes in the female. One may be able to do this by breeding the insects and working with the tissues of the larva or pupa. Judging from similar cases where the female number is known (for the Coleoptera, *Elatér* I, Fig. 229, Pl. 13, Stevens '06, and *Photinus pennsylvanicus*, figures not yet published; *Anasa tristis* and other Hemiptera, Wilson '05 and '06; *Pœcilopectera*, Fig. 283, Pl. 8 and Fig. 294, Pl. 9, Boring '07), we must suppose that the female number for *Diabrotica vittata* is twenty-two and for *Diabrotica soror* and *Diabrotica 12-punctata*, type I, twenty. Since the small heterochromosomes seem to be as likely to go to the spermatozoa which receive the odd chromosome (x) as to those which lack it, it would appear probable that the conditions with reference to the small heterochromosomes in the female are the same as in the male, and more-

over it is perfectly possible for more than four to occur in either male or female, as will be seen from the tables on p. 8.

DISCUSSION.

Sex Determination

For the present it is necessary to assume that the number of chromosomes in the female bears the same relation to the number in the male as in other cases among the Coleoptera and Hemiptera where an odd or unpaired heterochromosome is present in the male. The division products of the unpaired chromosome pass to one-half of the spermatozoa and these spermatozoa fertilize the eggs which develop into females; while the spermatozoa which lack the odd chromosome fertilize the eggs which produce males. This still seems to be as far as we can safely go in discussing the relation of the odd chromosome to sex determination. This chromosome is uniform in its behavior in the three species of *Diabrotica*, and it seems clear that it alone of the heterochromosomes described can have any connection with the determination of sex.

The "Supernumerary" Chromosomes

The small heterochromosomes in *Diabrotica 12-punctata* were first seen in some first spermatocyte spindles by Miss Anne M. Lutz of the Carnegie Institution of Experimental Evolution, Cold Spring Harbor, more than two years ago, but the matter was not followed up.

Prof. E. B. Wilson, in a recent communication (*Science*, n. s., vol. 26, no. 677, p. 870), has given the name "supernumerary" chromosomes to certain additional heterochromosomes in *Metapodius* (Hemiptera), and perhaps that name is as good as any other for the additional small heterochromosomes which appear in variable numbers in about 50 per cent of random collections of *Diabrotica soror* and *Diabrotica 12-punctata*. As in *Metapodius* the number of supernumeraries is constant for the individual. In *Metapodius* the supernumeraries are described as accompanying a pair of idiochromosomes with which they frequently unite to form a compound element in the second spermatocyte.

In the *Diabrotica*s they are present with a larger unpaired heterochromosome, and there is no evidence that they are ever united with it. The most puzzling characteristic of the supernumeraries in the *Diabrotica*s is the fact that they may in the same individual divide in either maturation division, and when two, three or four are present, each one may divide in either spermatocyte division, thus giving great diversity in the chromatin content of the spermatozoa. In *Metapodius* the supernumeraries are described as dividing in the first division.

Occasionally, as in Figs. 64 and 73 two of the supernumeraries seem to be paired in the metaphase of the first division, but this is probably accidental, as it is not constant in any individual.

The only other known case among the *Coleoptera* at all resembling this is that of the steel-blue flea-beetle, *Haltica chalybea*, which has a large and a small heterochromosome which are often widely separated in the metaphase of the first spermatocyte (figures not yet published). In the anaphase, however, the two heterochromosomes are found between the two daughter plates, and one goes to each second spermatocyte. This is merely a case of late pairing and the distribution of the division products of the two heterochromosomes to the spermatozoa is the same as in other cases of an unequal pair of heterochromosomes.

The first lot of *Diabrotica 12-punctata* were dissected out and all fixed together, so there was no opportunity to connect differences in the germ cells with differences in external characters of the insects, if such existed. In the California material obtained in December, 1906, and March, 1907, from Miss McCracken, each beetle, after dissecting out the testis or ovary, was preserved in alcohol, and later placed in the vial with its germ gland. In the December lot there was a difference of 1 mm. in the length of the Elytra, some measuring 4.5 mm., others 5.5 mm. All of the smaller beetles had the odd heterochromosome only, the others one supernumerary additional; and it was quite naturally supposed that there might be two distinct species or varieties, one of which had only the large unpaired heterochromosome, the other an unequal pair of heterochromosomes. In the March lot, one exception occurred—a small beetle had the additional small chro-

mosome, and there were several individuals of intermediate size which had one or more supernumeraries. In June, July and August, 100 males of *Diabrotica soror* were studied in California. The length of the elytron from origin to tip, measured to the nearest fourth of a millimeter in a straight line—not over the curve—was recorded for each beetle, the insects numbered and kept for future reference. After the hundred had been collected, measured and studied, they were arranged in series according to nuclear type. It was at once evident that the insects of type I and type II were about equally variable in size, and that all of the variations in fusion of spots occurred in each type. In fact no constant difference in external characters could be detected which might indicate two species. Thinking that possibly the variability in size might be different in the early and late broods, two lots of 100 each were collected November 1 and December 1, 1907, and measured without regard to the character of the germ cells. The results are given in a table below. Meanwhile 100 males of *Diabrotica 12-punctata* had been collected, measured, and the testes studied in aceto-carmin in October, 1907. This species is somewhat less variable than *Diabrotica soror*, but the two types with reference to the supernumerary chromosomes show the same kind of variations.

The variability in length of elytra of the different species and types is shown in Tables I and II.

TABLE I
Diabrotica soror.

Length of elytron in mm.....	3	3.25	3.5	3.75	4	4.25	4.5	4.75	5	5.25	5.5	5.75
Type I, ϕ s.....	1		4		14	4	16	2	6		1	48
IIa, 1 s.....			1		8	5	10	1	6	1	1	33
IIb, 2 s.....					5	2	6		2			15
IIc, 3 s.....							1		2			3
IId, 4 s.....							1					1
June 23 to August 7.....	1	0	5	0	27	11	34	3	16	1	2	100
November 1.....			1	1	7	15	22	20	27	6	1	100
December 1.....				1	9	9	25	17	23	11	4	100
Total.....	1	0	6	2	43	35	81	40	66	18	7	300

TABLE II
Diabrotica 12-punctata

Length of elytron in mm	3	3.25	3.5	3.75	4	4.25	4.5	4.75	5	5.25	5.5	5.75		
Type I, o s.....						2	7	7	23	9	1	2	51	
IIa, 1 s.....			1				7	4	13	2	7	1	35	
IIb, 2 s.....					1			1	9				11	
IIc, 3 s.....							1		1				2	
IId, 4 s.....								1					1	
Total.....					1	1	2	15	13	46	11	8	3	100

It will be seen from the tables that *Diabrotica soror* is somewhat more variable and averages smaller in early summer than in late autumn; also that there is a possibility of two or three intergrading groups. The latter fact would not, however, seem to have any significance with reference to the supernumerary chromosomes, since in *Diabrotica 12-punctata* (Table II) the curve of variability is very steep with one mode at 5 mm. The 100 specimens of *Diabrotica 12-punctata* included in Table II were collected on some late goldenrod in one corner of a field on October 3, 4 and 9; the first 100 in Table I, in one rose garden in small collections extending over about six weeks. In both lots, most of the insects had recently emerged, and the conditions of temperature and nutrition under which they had developed could not have varied very greatly.

The one significant result so far as the supernumerary chromosomes are concerned is the parallel series of numbers for the five types of the two species—*Diabrotica soror*, 48, 33, 15, 3, 1 and *Diabrotica 12-punctata*, 51, 35, 11, 2, 1. Were it not for this parallelism of results in the two similar but geographically widely separated species,¹ one might suppose the presence of the supernumeraries to be accidental, due perhaps to an irregularity in the breaking up of the spireme or to imperfect metakinesis somewhere in the history of the male or female germ cells. The behavior of the supernumeraries in the growth stages of the spermat-

¹ *Diabrotica 12-punctata* occasionally ranges into California, but belongs more especially to the eastern half of the United States, being perhaps most abundant in the Mississippi Valley.

cytes would suggest that they might have originated in a detached portion of the odd chromosome (x), but such a supposition is not borne out by their later behavior in the maturation divisions, nor is there any evidence of an unequal pair among the other chromosomes indicating accidental separation of a part of one chromosome.

The only evidence I have that the supernumeraries might be chromosomes in the process of development or degeneration is the one individual (*Diabrotica* 12-punctata, No. 83 of the lot of 100 collected in October, 1907) in which one very small supernumerary was observed (Figs. 76-78). In other cases there seemed to be remarkable uniformity in size without regard to the number present.

If at some period in the past history of the race before the eastern and western species separated one supernumerary arose in any way, its peculiar habit of division, sometimes in one, sometimes in the other maturation division, may have given rise to the proportional numbers of the different types in the two species. Or it may still be possible, as was surmised earlier in the study, that (1) there will prove to be two distinct types (varieties or species) in each of the present species, one having the large unpaired heterochromosome only, the other having an unequal pair of heterochromosomes like that in *Haltica*, and that (2) the irregularities in time of division and the consequent peculiarities in number and distribution of the supernumeraries in *Diabrotica* are to be attributed to hybridism. If this should prove to be true it would indicate little or no hereditary value for these supernumeraries or for the smaller members of the unequal pair in other Coleoptera. A careful biometrical study of several external characters may bring to light some differences which can be associated with the presence or absence of the supernumeraries. The only other difference in the chromosomes of the two types seems to be a variation in the form of the odd chromosome (x). In type I it is usually nearly spherical in growth stages, while in type II it is more or less elongated.

Until the material is investigated further, it hardly seems worth while to discuss at any greater length the hereditary significance of

the supernumerary chromosomes or the possible results of their irregular distribution. It however seemed advisable to publish the results which have been obtained, as considerable time must elapse before more material can be worked over; and it is to be hoped that another summer's work in California with breeding experiments and collections from different localities may furnish the data which are now lacking, and clear up the whole matter.

SUMMARY

1 *Diabrotica vittata* has twenty-one chromosomes, ten pairs and an unpaired heterochromosome which behaves like the odd chromosome in other Coleoptera and in the Orthoptera and Hemiptera homoptera, dividing in the second spermatocyte division, but not in the first. Synapsis occurs at the close of the synkinesis stage. A chromatin nucleolus is present in all of the spermatids.

2 *Diabrotica soror* and *Diabrotica 12-punctata* both have in all cases nineteen chromosomes, nine pairs and an unpaired heterochromosome, which divides like that in *Diabrotica vittata*. About 50 per cent of the individuals examined have only nineteen chromosomes, the remaining 50 per cent have from one to four additional or "supernumerary" chromosomes which divide in either spermatocyte division, not in both, and may therefore give rise to from four to ten different kinds of spermatozoa with reference to their chromatin content, in the same individual. The percentage of individuals containing no supernumerary chromosome, one, two, three, or four supernumeraries, is nearly the same for the two species—48, 33, 15, 3, 1 for *Diabrotica soror* at Mountain View, California, and 51, 35, 11, 2, 1 for *Diabrotica 12-punctata* at Bryn Mawr, Pa. It has not as yet been possible to associate the different nuclear types with variations in any external character.

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Bryn Mawr, Pa.

NOTE—A part of the facts concerning the chromosomes in *Diabrotica soror* were given at the International Congress of Zoölogists in Boston, August 21, 1907.

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DESCRIPTION OF PLATES

Figs. 1 to 48, 50, 54 and 66 to 71 were drawn from sections with 2 mm. obj. and 12 oc.; Figs. 49, 51 to 53 and 55 to 65 from aceto-carmin preparations with 2 mm. obj. and 6 oc.; Figs. 72 to 77 from aceto-carmin preparations with 2 mm. obj. and 12 oc. The magnification of all of the figures was then doubled with a drawing camera, and the plates reduced one-half.

Lettering used on Plates

x = the unpaired, "odd" or "accessory" chromosome.

n = the chromatin nucleolus of the spermatids.

s = a "supernumerary" chromosome.

s_1, s_2, s_3, s_4 = 1, 2, 3 or 4 supernumerary chromosomes in the same individual.

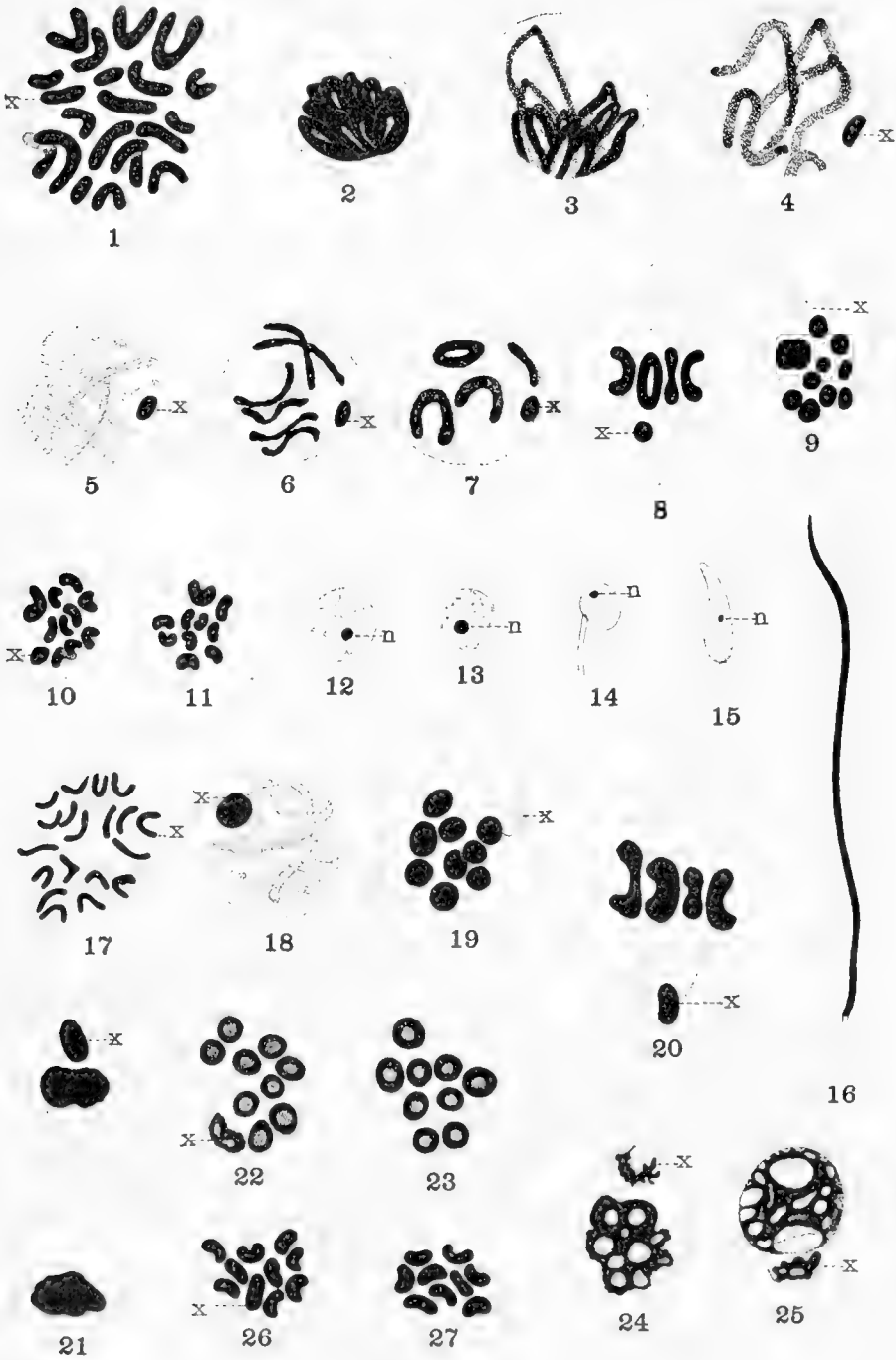
PLATE I

Diabrotica vittata

- Fig. 1 Spermatogonial metaphase, twenty-one chromosomes.
- Fig. 2 First spermatocyte, synizesis stage.
- Fig. 3 First spermatocyte, synapsis stage.
- Fig. 4 First spermatocyte, postsynapsis stage.
- Fig. 5 First spermatocyte, spireme stage.
- Figs. 6 and 7 First spermatocytes, prophase.
- Figs. 8 and 9 First spermatocytes, metaphase.
- Figs. 10 and 11 Second spermatocytes, metaphase.
- Figs. 12 to 15 Spermatids.
- Fig. 16 Ripe spermatozoön.

Diabrotica soror. Type I

- Fig. 17 Spermatogonial metaphase, nineteen chromosomes.
- Fig. 18 First spermatocyte, spireme stage.
- Figs. 19 and 20 First spermatocytes, metaphase.
- Fig. 21 First spermatocyte, anaphase.
- Fig. 22 and 23 First spermatocyte, daughter plates.
- Figs. 24 and 25 Second spermatocyte, rest stage.
- Figs. 26 and 27 Second spermatocytes, metaphase.



7 m 5 d

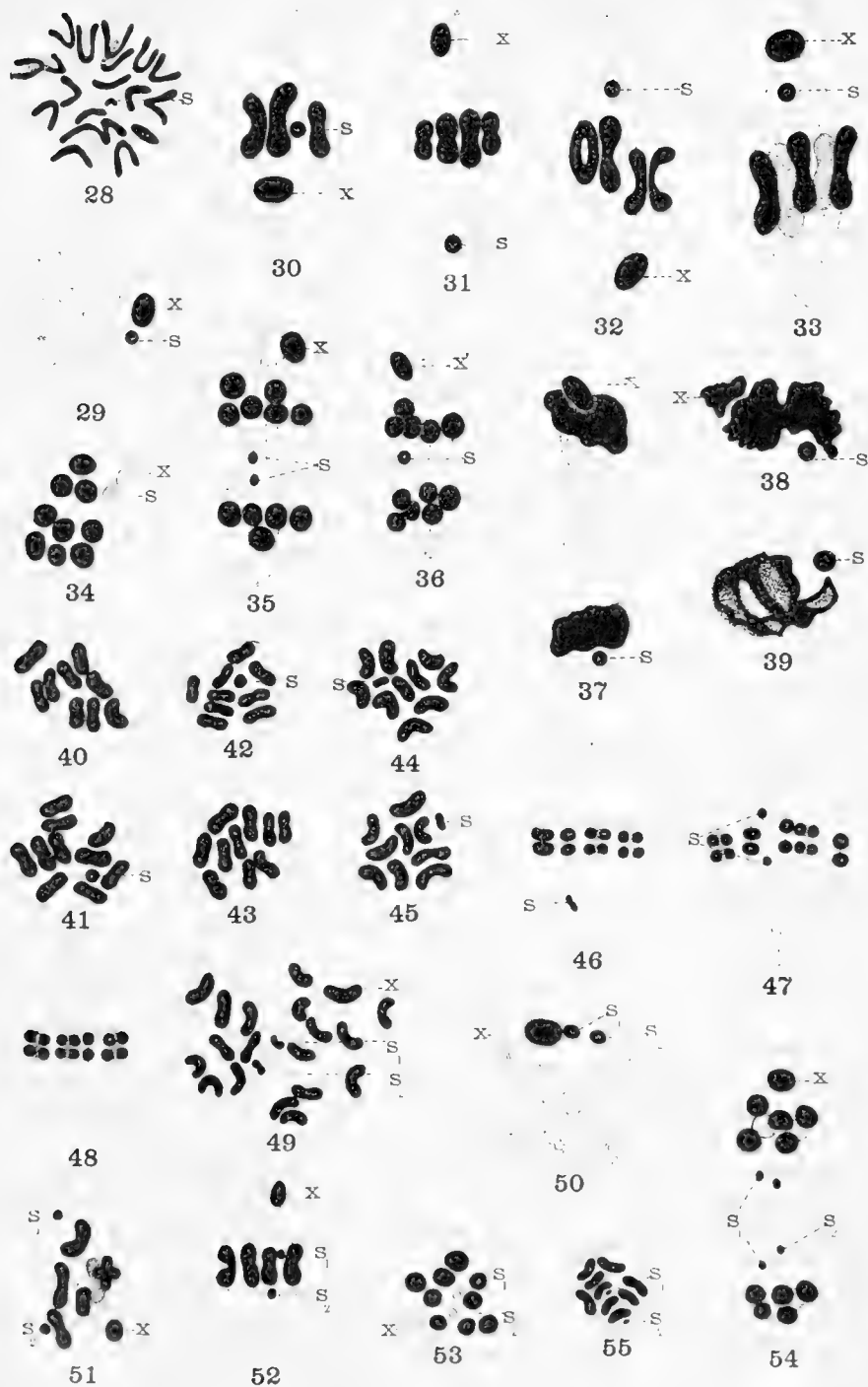
PLATE II

Diabrotica soror. Type IIa

- Fig. 28 Spermatogonial metaphase, twenty chromosomes.
Fig. 29 First spermatocyte, spireme stage.
Figs. 30 to 34 First spermatocytes, metaphase.
Figs. 35 to 37 First spermatocytes, anaphase.
Fig. 38 First spermatocyte, telophase.
Fig. 39 Second spermatocyte, rest stage.
Figs. 40 to 45 Second spermatocytes, metaphase, polar view.
Figs. 46 to 48 Second spermatocytes, metaphase, lateral view.

Type IIb

- Fig. 49 Spermatogonial metaphase, twenty-one chromosomes.
Fig. 50 First spermatocyte, spireme stage.
Fig. 51 First spermatocyte, prophase.
Figs. 52 and 53 First spermatocytes, metaphase.
Fig. 54 First spermatocyte, anaphase.
Fig. 55 Second spermatocyte, metaphase.



n m s del

PLATE III

Diabrotica soror. Type IIc

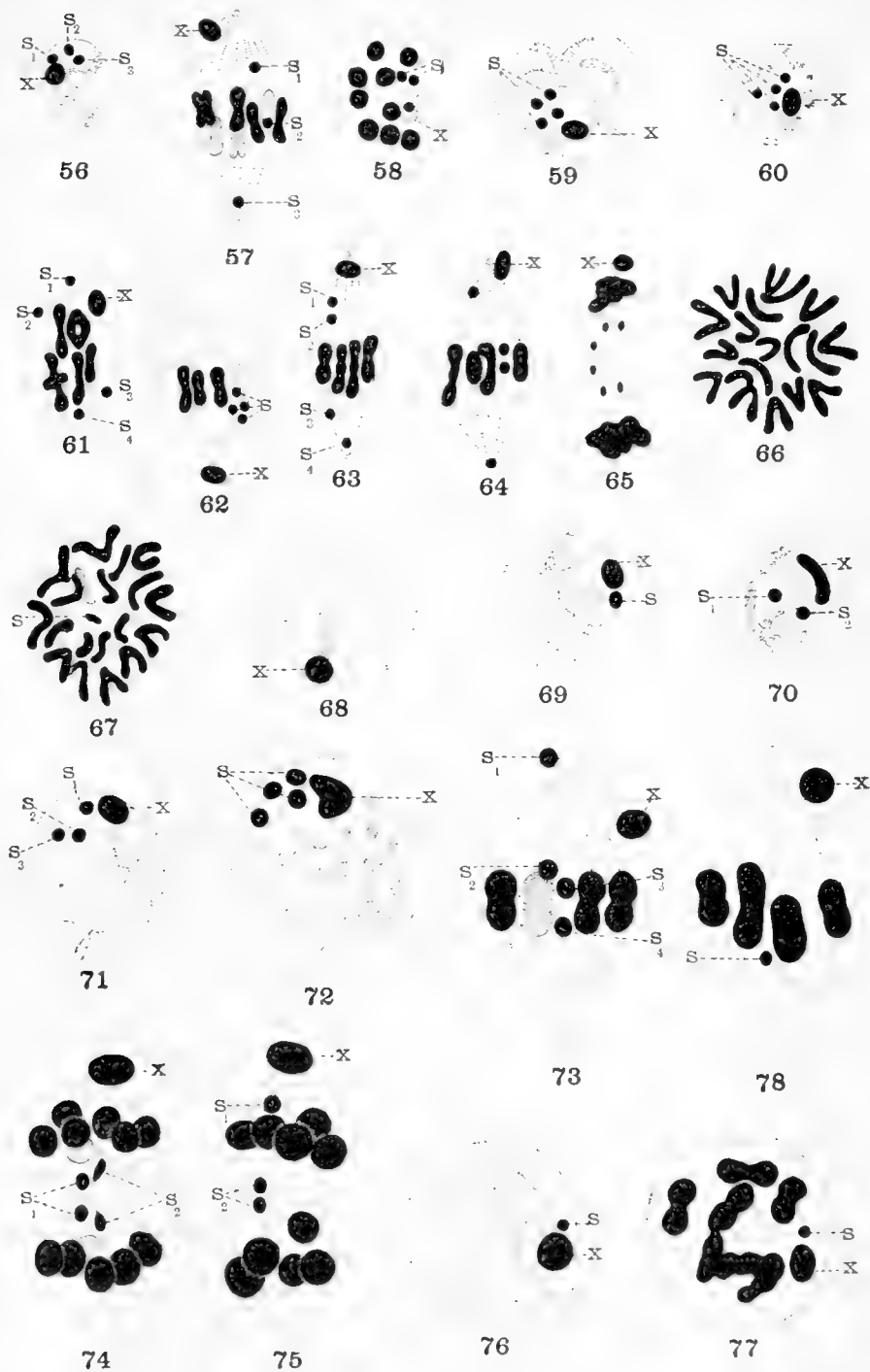
- Fig. 56 First spermatocyte, spireme stage.
Figs. 57 and 58 First spermatocytes, metaphase.

Type IIId

- Figs. 59 and 60 First spermatocytes, spireme stage.
Fig. 61 First spermatocyte, prophase.
Figs. 62 to 64 First spermatocytes, metaphase.
Fig. 65 First spermatocyte, anaphase.

Diabrotica 12-punctata

- Fig. 66 Spermatogonial metaphase, nineteen chromosomes.
Fig. 67 Spermatogonial metaphase, twenty chromosomes.
Fig. 68 First spermatocyte, spireme stage, no supernumerary.
Fig. 69 First spermatocyte, spireme stage, one supernumerary.
Fig. 70 First spermatocyte, spireme stage, two supernumeraries.
Fig. 71 First spermatocyte, spireme stage, three supernumeraries.
Fig. 72 First spermatocyte, spireme stage, four supernumeraries.
Fig. 73 First spermatocyte, metaphase, four supernumeraries.
Figs. 74 and 75 First spermatocytes, anaphase, two supernumeraries.
Figs. 76 to 78 First spermatocytes, unusually small supernumerary.



THE EXPERIMENTAL CONTROL OF ASYMMETRY AT DIFFERENT STAGES IN THE DEVELOPMENT OF THE LOBSTER.

BY

VICTOR E. EMMEL

INTRODUCTION

The asymmetry of decapod crustacea has recently been studied by Przibram ('01, '02, '05, '07), Morgan ('04), Zeleny ('05) and Wilson ('05). This asymmetry is manifest in the first pair of claws or chelæ—one of which is larger and frequently structurally different from the other. It has been found in some cases that if the large chela is removed, a small one may regenerate in its place. At the same time, the original small chela on the opposite side of the body may grow into a large one. This transposition of chelæ is known as "reversal of asymmetry." A complete reversal of asymmetry follows the amputation of the large chela in the adult of *Alpheus* as shown by Przibram and Wilson. On the other hand, such a reversal is not obtained in similar experiments with the adults of the hermit crab and lobster as found by Morgan and Przibram. This I have confirmed in regard to the lobster by experiments with over 200 adults, in none of which was there obtained a transposition of the chelæ.

The previous studies have dealt only with adult animals. In view of this fact it seemed desirable to investigate the establishment of asymmetry at various stages in the growth of the lobster—one of the forms in which reversal does not occur in the adult. This has been done with the results about to be described.

The work was carried on at the Experiment Station of the Rhode Island Commission of Inland Fisheries, and I desire to express my indebtedness to Dr. A. D. Mead for generously permitting me to

use the apparatus and the excellent material available at the lobster hatchery.

NORMAL DEVELOPMENT OF THE CHELÆ

A brief description of the normal development of the chelæ of the lobster may aid in understanding the nature of the present experiments.

In the adult lobster, one of the two chelæ, either the right or the left, is a rather long slender nipper claw, and the other is a larger and more massive crusher. Each claw consists of a movable jaw, the dactyl, and an immovable jaw or index. In very young lobsters the right and left chelæ appear alike. During the first three larval stages they are embryonic in character. The claws are relatively short and broad; the index is smaller than the dactyl and both index and dactyl are beset with long hairs or bristles. During the fourth and fifth stages, the claws have become long and slender but are still alike. Characteristic tactile hairs and pointed teeth appear and the claws now begin to resemble the adult nipper type.

At about the sixth stage however a divergence in the differentiation of the two chelæ becomes apparent. In one of the chelæ the nipper characters continue to develop. This claw retains the long slender form characteristic of the adult. Tactile hairs are distributed in a dense fringe on each side of the dentate margin. The pointed cutting teeth are arranged in a linear series for each jaw with the exception of a stout displaced tooth about midway in the dentate margin of the index. In marked contrast to this development of the nipper, the other claw becomes wider, broad tubercle-like teeth develop, and the tactile hair of the nipper type gradually disappears in successive moults. Thus the adult crusher claw comes to be characterized by the almost entire absence of tactile hairs, and the presence of broad crushing teeth; and by a form larger and more massive than that of the nipper. The final result is the establishment of the adult asymmetry.

In the development of the lobster therefore there is a series of larval and adolescent stages, in which there is a transition from

symmetrical to asymmetrical chelæ. In this asymmetry the crusher occurs as frequently on one side of the body as on the other.

PLAN AND METHOD OF THE EXPERIMENTS

The present experiments were made in the following stages of the lobster's development—the stages being designated as first, second, etc., according to the number of moults since the time of hatching:

The second larval stage;

Fourth stage;

Fifth stage;

Twelfth stage or lobsters a year old;

Adult lobsters.

All of these experiments attempt to determine to what extent asymmetrical differentiation of the chelæ can be controlled by amputation. In lobsters, as is well known, an injured limb is thrown off spontaneously or *autotomously*, separating along a certain "breaking plane" near the basal joint.

It was found necessary to exercise great care in the mutilation and rearing of the delicate larval lobsters. The chela was removed, under a small hand lens when necessary, by grasping the tip of the limb with a pair of forceps. In the older lobsters the chela promptly separates at the breaking plane. In the younger lobsters however the separation is not so readily obtained and a gentle pull may be required. The most difficult period in which to keep the lobsters alive is during the second and third stages. After several failures with ordinary aquaria the best results were obtained by keeping the animals in a current of fresh sea-water. This was accomplished by means of a rather elaborate apparatus built in the pool of a wooden float. The bottoms of pulp pails were removed and replaced by "bobbinet" cloth with meshes small enough to prevent the escape of the lobsters. A second cover, or false bottom, of mosquito bar was also found necessary—not to confine the lobsters but to prevent the ever present shrimp from pulling them out through the meshes. These pails were then suspended in the water of the pool. In

each pail was placed a small paddle not unlike a boat propeller, consisting of a vertical shaft with two horizontal blades at its lower end. Each paddle was kept in motion by proper gearing with a "live shaft." The blades were beveled so as to give an upward movement to the current of water. In this way it was possible to rear a small per cent of the mutilated lobsters through the critical larval stages.

After the fourth stage, the lobsters were placed in a floating car divided into small compartments. Each lobster was kept in a separate compartment and a careful record made of its mutilations, moults and regenerations.

EXPERIMENTS WITH LOBSTERS IN THE SECOND STAGE

On July 24, 1906, two groups of lobsters were mutilated. These lobsters had all hatched from the egg within about four days. In Group A, the right chela, and in Group B, the left chela was removed from each specimen. In spite of an exceedingly great mortality, thirteen specimens were reared beyond the sixth stage. After each moult the regenerated chela was invariably removed. Thus the limb on the opposite side was given a great advantage for growth in order to learn whether this chela could be made to differentiate into a crusher. The results are shown in the accompanying table. This table includes also the data from a supplementary experiment made during the following summer. In this experiment great difficulty was likewise experienced in rearing the mutilated animals, for out of 200 larval lobsters from which the right chela was removed, only three specimens lived beyond the sixth stage.

From the data for these sixteen lobsters it will be observed that when the chela had differentiated far enough to display asymmetrical characters, the claws which regenerated after amputation were all nippers; at the same time, the claws which were not mutilated, being thus given the greater advantage in growth, were all crushers.

TABLE I.

*Larval lobsters in the second stage. Original chelæ symmetrical.**Group A Right chela removed*

No.	Date of first moult	Number of subsequent moults	FINAL ASYMMETRY OF CHELÆ		
			Date	Right	Left
1	July 24, 1906	six	Sept. 29, '06	nipper	crusher
2	24, 1906	six	Oct. 6, '06	nipper	crusher
3	24, 1906	six	Sept. 29, '06	nipper	crusher
4	24, 1906	six	Nov. 8, '06	nipper	crusher †
5	June 12, 1907	four	Aug. 2, '07	nipper	crusher †
6	12, 1907	four + (*)	Sept. 21, '07	nipper	crusher
7	12, 1907	four +	Sept. 21, '07	nipper	crusher

Group B Left chela removed

8	July 24, 1906	six	Oct. 27, '06	crusher	nipper
9	24, 1906	six	Sept. 29, '06	crusher	nipper
10	24, 1906	six	May 31, '07	(?)	nipper †
11	24, 1906	six	Oct. 19, '06	crusher	nipper
12	24, 1906	six	Oct. 19, '06	crusher	nipper
13	24, 1906	six	Oct. 19, '06	crusher	nipper
14	24, 1906	six	Oct. 19, '06	crusher	nipper
15	24, 1906	six	July 12, '07	crusher	nipper ‡
16	24, 1906	six	Sept. 22, '06	crusher	nipper

* In specimens Nos. 6 and 7, the regenerated right chela was not removed after the moult to the sixth stage on July 14, and on account of unavoidable absence, a record of further moults was not kept.

† These specimens, unfortunately, died before there was a clearly developed asymmetry of the chelæ. In Nos. 4 and 5, the general appearance of the left claw and the characteristic double tubercle dentition at the base of the jaws indicated that these claws were differentiating into crushers. No. 10 however died on May 31 without having differentiated asymmetrically.

‡ It may be of interest to note that No. 15 was much slower in its differentiation than the other specimens. At the close of the experiment in 1906, this animal showed no evidence of asymmetry. It was kept through the winter and after three more moults during the following summer, this lobster, in harmony with all the others in Group B, developed a crusher claw on the right chela.

LOBSTERS IN THE FOURTH STAGE

At the fourth stage the lobster has made a marked advance toward the adult form. The chelæ however are still alike and symmetrical.

On July 25, 1907, seventeen specimens were mutilated on the day following the moult to the fourth stage. Each lobster was mutilated by the autotomous removal of the right chela. The results are shown in Table II.

TABLE II

Lobsters in the fourth stage. Original chela symmetrical. Right chela removed

No.	Stage	Mutilation	Stage	Mutilation	Moult to Stage VI	FINAL ASYMMETRY OF CHELAE	
						Right	Right
1	Moulted to the fourth stage, June 24.	Removed right chela, June 25.	Moulted to fifth stage, July 7 and 8.	Removed regenerated chela, July 11.	July 16	nipper	crusher
2					16	nipper	crusher
3					16	nipper	crusher
4					16	nipper	crusher
5					16	nipper	crusher
6					16	nipper	crusher
7					16	nipper	crusher
8					16	nipper	crusher
9					(?)*	nipper	crusher
10					(?)	nipper	crusher
11					(?)	nipper	crusher
12					(?)	nipper	crusher
13					(?)	nipper	crusher
14					(?)	nipper	crusher
15					(?)	nipper	crusher
16					(?)	nipper	crusher
17					(?)	nipper	crusher

* Specimens 9 to 17 moulted to the sixth stage a few days after July 16, but the date of moult was not recorded.

It is readily seen that these results show a marked uniformity. Without exception the mutilated claws became nippers; the claws which were not mutilated became crushers.

LOBSTERS IN THE FIFTH STAGE

The fifth stage is especially important because, normally, at the next moult asymmetrical characters are displayed. Consequently, during this period there must be a rapid progress in the differentiation of the chelae.

The lobsters were mutilated by the autotomous removal of the right chelæ July, 1907. The mutilations were made about four days after the moult to the fifth stage. Through the kindness of Dr. A. D. Mead and his assistant, Mr. L. N. Wight, some of the lobsters were kept alive until the final data could be obtained in September. The results are shown in Table III.

TABLE III

Lobsters in the fifth stage. Original chelæ symmetrical. Removed right chela four days after moult to fifth stage July, 1907

FINAL ASYMMETRY, SEPTEMBER 21			
No.	Stage *	Right chela	Left chela
1	seventh (?)	nipper	crusher
2	seventh (?)	nipper	crusher
3	seventh (?)	nipper	crusher
4	seventh (?)	crusher	nipper
5	seventh (?)	crusher	nipper
6	seventh (?)	crusher	nipper
7	seventh (?)	crusher	nipper
8	died		
9	died		
10	died		

* By September 21, these lobsters were apparently all in the seventh stage, although the stage cannot be positively stated because a record of all the moults was not obtained.

These results are in marked contrast with those obtained for the preceding stages. Instead of all the uninjured claws producing crushers, they produced three crushers and four nippers. At the same time, the regenerated claws, instead of being all nippers include three nippers and four crushers. Since in the adult lobster the crusher appears about as frequently on one side of the body as the other (and this is equally true of the nipper), it appears that the normal development was not modified by the removal of one chela. Evidently therefore during the fifth stage, in which the chelæ are apparently still symmetrical, the controlling influence of such amputations upon symmetry disappears.

A point which should receive further investigation for this stage is the time of mutilation with reference to the moult. It will be observed that in the above experiments the mutilations were made several days after moulting. In another experiment the left chela was removed from a number of lobsters on the day in which they had moulted to the fifth stage. Only four of the specimens lived until the chelæ displayed asymmetrical characters, but it is interesting to note that for each lobster, a crusher developed on the right or uninjured chela, and that the regenerated claw was a nipper. This result indicates that possibly the asymmetry may be controlled at this stage, provided that the mutilations are made sufficiently early.

IMMATURE LOBSTERS, A YEAR OLD

With the assistance of Mr. E. W. Barnes, superintendent of the Experiment Station of the Rhode Island Fish Commission, we succeeded in keeping about thirty-five lobsters, hatched in July, 1905, until the following summer. At this time these yearling lobsters were all in about the twelfth stage and averaged two inches in length. The asymmetry of the chelæ is clearly developed at this age. But when it is recalled that the lobster does not attain sexual maturity until about the fifth year, it will be readily appreciated that these yearling lobsters were still quite immature and at a period of rapid growth. It seemed desirable therefore to ascertain the degree of stability which the asymmetry may have attained at this age as compared with the adult. The experimental results obtained are shown in Table IV.

In this experiment on yearling lobsters, 15 were mutilated by the autotomous removal of the crusher chelæ (Group A), and 14 were mutilated by the removal of both chelæ (Group B). In both groups the regenerated claws were again amputated. In no case in either group did these mutilations and consequent regenerations reverse the original asymmetry. Each yearling lobster retained its original right or left handed arrangement of the chelæ.

It should be added however that in the case of the crusher

chela the regenerating claw is not always a characteristic crusher from the outset, but frequently displays, at first, characters intermediate between those of a crusher and a nipper.¹

TABLE IV

*Lobsters a year old. Original chelæ asymmetrical. Group A Crusher chela removed**

LOBSTERS	ORIGINAL RELATION OF CHELÆ		RELATION OF CHELÆ AFTER TWO REGENERATIONS AND TWO MOULTS	
	Right	Left	Right	Left
8 specimens	crusher	nipper	crusher	nipper
7 specimens	nipper	crusher	nipper	crusher

*Group B Both chelæ removed**

8 specimens	crusher	nipper	crusher	nipper
6 specimens	nipper	crusher	nipper	crusher

* After the first moult the regenerated chelæ were again removed from each lobster.

ADULT LOBSTERS

Przibram ('01, '02) and Morgan ('04) have already observed that in adult lobsters a typical reversal of asymmetry as the result of amputation and regeneration of the chelæ has not been found. In the course of my experiments over 200 adults were mutilated by removing one or both chelæ. In no case did a crusher develop on the side which had originally carried a nipper, and the same was true, vice versa, for the nipper. As in the yearlings, but not to the same extent, the regenerating crusher chela is not always at first distinguishable as such, but may present characteristics intermediate between the nipper and crusher (Emmel, '06²). Also in certain very rare cases, symmetrical chelæ of either the nipper or crusher type may regenerate in place of the amputated asymmetrical limbs (Emmel, '06³, '07².) The fact to be emphasized however is that in these adult lobsters, the amputation of

¹ Compare Emmel '06² and Przibram '07, p. 291.

neither one nor both chelæ produced a reversal of the original asymmetry.

DISCUSSION

Until recently the phenomenon of reversal of asymmetry or compensatory regulation, which Przibram and Zeleny found in *Alpheus*, was not supposed to occur in such forms as the lobster or hermit crab. It appeared that these species were characterized by a "direct regeneration" of the original asymmetry. But the discovery that under certain conditions the adult lobster might regenerate a crusher from the stump of the amputated nipper chela (Emmel '06³) demonstrated that in the lobster, at least, both sides of the body might still retain the potentiality for the more highly differentiated type of crusher claw. The present results which show that asymmetrical differentiation can be controlled at early stages of development in the lobster, suggest that similar relations in the development and stability of asymmetry may be found in other crustacea.²

Various theories have been advanced concerning the factors which determine right or left asymmetry, and which may be discussed on the basis of the preceding experiments.

Herrick ('05) studied the shrimp *Alpheus*, and concluded that asymmetry of the chelæ in *Alpheus* and also in the lobster "is probably one of direct inheritance, all members of a brood being either right or left handed. That is to say, the normal position of the toothed or crushing claw is not haphazard, but is predetermined in the egg" (p. 225).

Conklin ('03, '05), without discussing inheritance, shows how inverse symmetry may be determined in the egg. He found reason for believing that the cause of inverse symmetry, which occurs regularly among some species and occasionally among all, man included, is to be found in the inverse organization of the egg, and

² It is interesting to find this suggestion already anticipated by Przibram. In his important monograph published in the *Archiv. f. Entw.-Mech.*, Bd. 25, 1907, p. 310 (received while the present paper was being written), he discusses the question, "Is the possibility of the reversal of chelæ present in those forms which have hitherto shown no reversal?" He concludes that in some of these (*Callinassa* and *Carcinus*) the asymmetrical relations may be altered.

that this inverse organization may be due to the maturation of the egg at opposite poles in dextral and sinistral forms ('05, p. 10). On this basis, the alternate appearance of right or left asymmetry in the lobster might be regarded as cases of inverse symmetry "resulting from slight alterations in the localization of germinal substances in the unsegmented egg."

Morgan ('07) does not venture to decide between the possibilities of "inheritance" and the structure of the egg, as determining right or left handedness in various species. He says "both possibilities seem to exist in the egg; but whether this can be referred to alternate dominance and recession, or to purely local conditions that arise during segmentation, is unknown" (p. 165).

It is evident that the present experiments at least demonstrate that the asymmetry of the adult lobster is not necessarily inherited nor even predetermined in the egg. However, the question still remains as to what factors in normal development determine right or left asymmetry. No evidence was found that the occurrence of right or left asymmetry in the lobster can be referred to germinal units having "alternating dominance and recession." The fact that in early development a crusher can be produced on either side of the body by the amputation of the opposite chela, indicates that the factors which control asymmetry become operative after hatching. What these factors are, or how they may be released by the amputation of a limb, is not known. We can merely refer to the fact that in early stages of development of the lobster the asymmetry of the chelæ can be experimentally controlled. When asymmetry has once been normally established, similar experiments no longer reverse it.

That the accidental loss of a limb in the young lobster may play an important rôle in determining the asymmetry of the adult is not improbable. For the autotomy of a chela during the exigencies of moulting or as the result of injury, is a common occurrence, especially among young lobsters. In an examination of several thousands of fourth stage lobsters it was found that a large per cent of the animals had lost either the right or left chela. In these the right or left asymmetry would not be inherited or due to the structure of the egg.

Przibram ('07), in his recent extensive work on "Die Scherenumkehr bei decapoden Crustaceen," found experimentally that a reversal of chelæ could be obtained in six genera and eleven species of crustacea, including forms in both the Macrura and Brachyura. He then inquires whether this capacity for reversal of asymmetry is a constant characteristic for a given species. Here he finds that the statement that a reversal of asymmetry always follows the amputation of the crusher ("*K - schere*") in a crustacean requires modification. For it appears that the readiness with which reversal occurs varies inversely with the size of the animal. Specimens of *Athanas*, *Alpheus*, *Typton*, *Callianassa*, *Carcinus* and *Portunus*, which are under 10 mm. in carapace length, showed a quick and complete reversal of asymmetry. But, on the other hand, the larger specimens showed a decrease in the tendency toward transposition of the chelæ, so that when the large chela is removed the chela on the opposite side retains its original form.

It appears therefore that the relations found in the control of asymmetry at different stages of development in the lobster, are also true for other crustacea with asymmetrical chelæ. For both in the lobster and in the forms described by Przibram, the various stages in their development form a series beginning with a complete control of asymmetry and ending with the disappearance of such control. In other words, the possibility for experimental control and reversal of asymmetry seems to be correlated in some way with the degree of differentiation or development, so that the greater the degree of development the more stable is the asymmetry of the organism.

SUMMARY

1 In the first four stages of the lobster's development, a crusher may be produced on either the right or the left side of the body by the autotomous amputation of the chela on the opposite side—the regenerated chela becoming a nipper.

2 During the fifth stage, although the chelæ are apparently still symmetrical, the possibility for such experimental control disappears.

3 In later stages of development, when the asymmetry of the chelæ has become established, the amputation of one or both chelæ does not produce a reversal of the original asymmetry.

4 The results of these experiments indicate therefore that the factors which control the asymmetry of the lobster become operative after hatching and are correlated with conditions of growth after the organism leaves the egg. No indication was found that the occurrence of right or left relations of asymmetry in this species can be referred to germinal units having "alternating dominance." It appears also that these relations are not due to an "inverse organization of the egg," for it is evident that up to the fifth stage right or left asymmetry can be produced at the will of the experimenter.

January, 1908

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THE PHYSIOLOGICAL BASIS OF RESTITUTION OF LOST PARTS

BY

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WITH ONE FIGURE

In a series of "Studies on Regulation" which have appeared in Roux's Archiv and the Journal of Experimental Zoölogy during the last five years, and in certain other papers (Child '06a, '06b), I have attempted to point out the essentially functional character of form-regulation and have defined regulation in general as a return or approach to physiological equilibrium after such equilibrium has been disturbed or altered (Child '06a). According to this idea form-regulation and functional regulation are both essentially the same thing. It is perhaps unnecessary to state again here what I have repeatedly stated, viz: that the term "functional" is used in this connection in its widest sense as equivalent with "dynamic" or "physiological" and so includes all dynamic factors in organic life. In other words, the problem of form-regulation is a physiological problem and not a problem *sui generis* as Driesch and various other authors have maintained.

Let us consider for a moment what these assertions imply as regards the factors concerned in the determination of any particular structure. If we assert that a given structure is altered or determined by functional conditions does not this assertion necessarily involve the idea of relation to its environment, intra-organic or extra-organic or both? As a matter of fact the very essence of the term "functional" as employed in these papers is to be found in the interrelation or correlation between the different parts of the organism and between the organism and its extra-organic environment.

That this could fail to be evident to any reader of these papers had not occurred to me until a recent paper by Prof. S. J. Holmes (Holmes '07) came to my notice. This paper is a restatement of the author's symbiotic theory of form-regulation and a reply to certain criticisms of my own (Child '06a) of an earlier statement of this theory (Holmes '04). Holmes maintains that my suggestions concerning the nature of form-regulation do "not contain any general principle of explanation for that functional substitution and equilibration upon which it is assumed that form-regulation depends. But I suspect that when his theory comes to be developed so as to supply this missing element it will involve the assumption of some such symbiotic relation between the parts of the organism as I have assumed" (p. 424). If I understand this assertion, it involves a serious misapprehension of my position. I have insisted again and again in my work on form-regulation in the interrelations or correlations between parts—in fact, certain of my papers have been concerned chiefly with showing that such relations existed. Moreover, it is in consequence of the existence of such relations that I regard form-regulation as essentially a functional process. Even in my earliest papers positive statements on this point were made. Thus, for example, on p. 219 of No. I of my *Studies on Regulation* (Child '02) in a consideration of the general body-form of *Stenostoma* I wrote: "Every organism is what it is because of the relation of all its parts to each other and to the rest of the world. If any of these relations are changed the organism is changed." And again in No. IV of the *Studies* (Child '04a) in a discussion of "formative factors:" "All the complex activities of which organisms are capable are 'formative factors:' when we can view all of these in their complex interrelations, then and then only shall we 'understand' organic form." Also in No. V (Child '04b): "The factors of organic form include all the activities of organic substance as well as the environmental factors in varying degree. Indeed, in most cases, if not in all, we may regard organic form as the visible effect upon the protoplasm of functional factors in the widest sense" (pp. 468-469). In the later papers these interrelations are still more strongly emphasized. I have preferred not to designate them as symbiotic relations since

I cannot see that anything is gained by the use of this term. Moreover, it seems to me that many of the correlations are not really symbiotic at all, except in so far as they may be mutual with respect to complex parts of the organism. For example, the mere mechanical union with other parts is undoubtedly in many cases one factor in preventing parts of the organism from undergoing regulation into wholes. But I cannot see that it serves any useful purpose to call such factors symbiotic relations. It seems preferable therefore to maintain that these relations, or as I believe we may more properly call them, correlations, are physically and chemically of all sorts possible in the material and environment in which they exist. Moreover, while many of them are undoubtedly mutual, i. e., reciprocal, at least as regards complex parts, others are just as certainly largely or wholly one-sided so far as form is concerned. It seems scarcely necessary to enlarge further upon this point. As regards the existence of relations between parts as an essential feature of regulation Holmes and I agree perfectly. As regards form-regulation we differ, in that it seems to me difficult or impossible to account for the facts on the basis of symbiotic relations, even in the widest sense.

Holmes' illustration of the process of regeneration is as follows: "Let us imagine an organism made up of a number of differentiated cells, each of which derives some advantage from some substances produced by the contiguous cells, and giving out some substance upon which the contiguous cells are more or less dependent. We will suppose that in addition to these differentiated cells, there are scattered through the body numerous indifferent or embryonic cells whose multiplication is held in check by the others, but which upon the removal of any part respond to the functional disturbance by growth and multiplication near the place of mutilation. We may represent our organism by the following diagram in which the differentiated cells are represented by the larger circles *A*, *B*, *C*, etc., and the indifferent cells by the smaller circles between them. Each cell such as *A* contributes something utilized by *B*, *G*, and *F*, and derives something in return from each of these sources. Now suppose *A* is removed: the indifferent cell lying nearby, no longer held in check by the same stimuli,

begins to grow and develop. What line of differentiation will it most naturally take? Owing to the symbiotic relation existing between the cells differentiation in the direction of *A* will be most favored as this secures it the advantages which *A* received. In other words, this will be the direction of development along which social pressure will tend to guide it. And the result will be a regeneration of the missing part" (Holmes '04, p. 282; '07, pp. 420, 411).

In 1906 (Child '06a) I criticised this illustration on the ground that if the cells or parts were mutually dependent, i. e., if symbiotic relations existed between them, removal of any one of them, e. g., *A*, would bring about changes in the others in consequence of which their influence upon the undifferentiated cell substi-

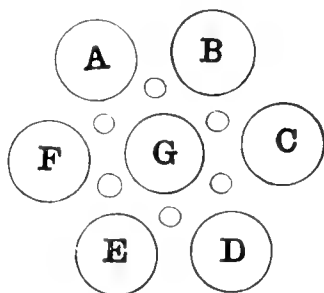


FIG. 1

tuted for *A* would be different from what it was originally, and hence the undifferentiated cell or part would develop—not into another *A* but into something else. There is logically no escape from this conclusion. The removal of *A* results in the formation of a new system different from the original and must necessarily do so, except under certain limiting conditions to be discussed below.

Holmes' reply to my criticism is as follows: "According to Child, since the removal of *A* would alter *B*, *G*, *F*, etc., not only something different would be developed in place of *A*, but the whole complex, according to my theory, would be profoundly altered. How far this tendency will result in a modification of these cells depends on the plasticity of the organism and the degree

of mutual dependence of the parts—factors of course which vary in different organisms. But Child overlooks the fact that according to the symbiotic relation assumed, the other cells *C*, *D*, *E*, etc., tend to keep *B*, *F*, *G* in their original condition. In so far as these remain in their original state, their influence on the indifferent tissue in the region of *A* will tend to mold it in the direction of the missing parts. In so far as *B*, *G* and *F* are modified through the loss of the missing part, their influence on the tissue in the region of *A* will come to be modified, and they will in turn modify the cells lying next to them. But, as there is a tendency for the modifications produced by the loss of *A*, to spread successively to other parts, there is also a tendency, according to my theory, toward the checking and reversal of this process. If the loss of *A* tends to modify *B*, *F* and *G*, the presence of *E*, *C* and *D* tends to hold them in place, and in so far as these are maintained through this influence they tend to mold the tissue in the position of *A* into the form of the missing part; and in so far as this is so molded, its modifying influence on *B*, *F* and *G* is diminished” (Holmes '07, pp. 425, 426).

I am unable to see that this argument shows that something like *A* may be generally replaced. Undoubtedly the modifying influence of *A* upon the contiguous cells or parts *B*, *F*, *G*, is lessened by the presence of other cells or parts, *E*, *C*, *D*, but it is not reduced to zero in any case where the relations between parts are mutual. The balance between the “tendency for the modification produced by the loss of *A* to spread” and the opposite tendency simply determines how great or how small the modification shall be. Something more or less like *A* may undoubtedly be produced in many cases, but according to this hypothesis we should expect that the regenerated part would differ more or less widely from the original part in most cases.

In fact, if the restoration of a part removed is purely a matter of interrelation between the various parts of the system, we must modify this hypothesis in either one of two ways to account for it. First: we may assume that the removal of the part, *A* in Holmes' diagram, does not alter the other parts, *B*, *G*, *F*, etc., in any way which affects essentially their interrelations with the parts of the system.

In this case the undifferentiated material which in the absence of *A* is stimulated to develop, will develop into another *A*. But in this case the relation between the original *A* and the other parts of the system is essentially one-sided and not mutual or symbiotic. Or as a second possibility, we may assume that the relation between *A* and the other parts of the system is such that removal of *A* produces modifications in the other parts only very slowly, while in the absence of *A* these other parts affect the undifferentiated cells in such manner as to bring about rapid development so that restoration is complete before the parts *B*, *G*, *F*, etc., have been appreciably altered by the absence of *A*. Here the relations, though in the final analysis mutual, are so far as *A* and its restoration are concerned, one-sided. In short, if we accept the symbiotic theory as a basis, we can account for the restoration of a part like that removed only by additional assumptions, according to which the relations involved in the restoration become practically one-sided rather than mutual.

In my earlier criticism of Holmes' theory (Child '06a, pp. 420, 421) the following statement appears: "To return to Holmes' diagram, replacement of *A* can occur only when the relation is largely one-sided, i. e., when *A* is dependent on *B-F*, but these latter are not to any marked degree dependent on *A*. In this case, and in this case only, will the "social pressure" force the undifferentiated cell to differentiate into something like *A*."

Holmes replies to this: "Where redifferentiation from new tissue is concerned, as in the present case, it is not the relation of *A* to *B-F*, that should be more or less one-sided, but the relation of the tissue in place of *A* to this complex. This is an important distinction which Child does not seem to have considered. *B-F* are relatively fixed, the tissue in place of *A* is young and plastic, and more dependent so far as the direction of its differentiation is concerned, upon *B-F*, than these are upon it. We may grant that when regeneration occurs, the relation of dependence between the old parts and the new tissue is more or less one-sided, although the relations of the part removed may not have been. This would naturally result if the parts were relatively stable. They may be in a symbiotic relation, nevertheless, each part contributing in

some way to the normal functioning of the others, and dependent to the extent that the removal of one part may alter only to a certain degree the quality and quantity of the activity of the surrounding parts, without producing extensive modification of structure or function" (Holmes '07, pp. 426, 427).

The first part of this argument seems to me to obscure the real point at issue. If the relation between A on the one hand and $B-F$ on the other is not at least largely one-sided, removal of A must alter $B-F$, and if, as Holmes assumes, the new tissue which replaces A is more dependent on $B-F$ than they on it, it becomes still more difficult to understand how the new tissue can replace A , for, so far as $B-F$ are concerned, it does not at first take the place of A functionally. In the last sentence quoted, Holmes attempts to save his symbiotic theory after admitting that in regeneration the relation may be more or less one-sided, by suggesting the existence of symbiotic relations which do not produce "extensive modifications of structure or function" when one part is removed. It seems to me that such relations are negligible quantities so far as form-regulation is concerned, for if removal of a part of the complex does not produce extensive modifications of structure or function in the parts remaining, we must certainly conclude that the presence of this part is not essential for the maintenance of the characteristic structure and function in the other parts. Evidently then this assumption does not relieve us from the necessity of assuming that the remaining parts are, so far as form and structure are concerned, practically independent of the part removed, i. e., that the relations involved in form-regulation are largely one-sided in cases where restoration of the missing part occurs. It makes no difference whether we regard the persistence of $B-F$ in essentially unchanged condition after the removal of A as due to "relative stability" or to real independence of A . The fact remains that A can be restored only in case the other parts do persist essentially unchanged during the period between its removal and its restoration to a certain stage of development. And it is just as certain that Holmes' symbiotic hypothesis cannot account for such persistence except by assuming the existence of special conditions which modify the relations between parts so

that they become essentially one-sided rather than mutual. If the restoration of a part like that removed were the exception rather than the rule, or even if it were less frequent, we might still accept the hypothesis. But a hypothesis which can account for the typical phenomena within its field, only with the aid of additional special assumptions, which in this case amount practically to throwing over the hypothesis, can scarcely be regarded as satisfactory.

The numerous cases already known where an animal is capable of replacing a part repeatedly after successive removals seem to me to furnish additional evidence against Holmes' theory. Even so important a part as the head may be replaced repeatedly in many forms without appreciable change in character. It is scarcely probable, to say the least, that the relations between the head-region and other parts are one-sided in the sense that it is dependent on them, while they are independent of it. And if the relation is not one-sided in this sense, we should expect that repeated removals of the head would bring about essential changes in the other parts, even if the first removal did not. If such changes in the other parts do occur to any marked extent, it is difficult to understand how a new head like the old can be replaced time after time as the result of "social pressure," for such changes in the old parts must alter the character of the "social pressure." Here then Holmes' theory leads us into something closely approaching a dilemma.

Holmes continues: "If the parts *B-F* were more plastic, absence of *A* would naturally tend to cause greater changes in them, especially if new tissues were not produced in place of *A*, which would come to assume some of the missing functions before the modification extended very far. There would then be a progressive modification extending from the region of *A*, which would tend to become less the farther it extended, but eventually perhaps affecting more or less the entire organism. Functional equilibrium would then be maintained by working over the organism so that all the parts were adjusted to functioning on a smaller scale. The different methods of regulation, through morphallaxis, regeneration and the various combinations of these proc-

esses are, I believe, interpretable according to the symbiotic theory, and the relations of regeneration and morphallaxis to the degree of specialization of the parts which Child has elaborated, are, in fact, exactly what the theory would lead us to expect" (Holmes '07, p. 427).

Here Holmes fails absolutely, so far as I can see, to explain how and why equilibrium will be maintained. Certainly the "progressive modifications" resulting from the removal of *A* cannot bring the system back to its original condition: they must lead either to destruction of the system, or rather of the parts of it which remain, or else to a new condition of equilibrium different from the old. How does Holmes know that "functional equilibrium would then be maintained by working over the organism so that all the parts were adjusted to functioning on a smaller scale?" What factor in the parts remaining compensates for the "progressive modifications" resulting from the loss of *A*? Why should there be any compensation? To none of these questions does Holmes' hypothesis give any answer.

According to the symbiotic theory as Holmes has presented it, the removal of a part is, at least in many cases, analogous to removal of a quantity from one side of an equation without change in the other. It is obvious that such procedure alters the value of one side of the equation in all cases except where the quantity removed is equal to zero.

In short I believe that Holmes' theory of regulation overlooks the most essential feature in the process of replacement of a part removed. This feature is the qualitative functional totipotency of the remaining parts after removal of the part in question. In other words, a part which has been removed cannot be replaced unless something remains after its removal which plays its part functionally in some degree. According to Holmes' theory its place is taken by undifferentiated tissue, which is forced to develop into something like the part removed by the influence exerted upon it by other parts. But this undifferentiated tissue cannot exert the same influence on other parts as was exerted by the part removed. Moreover it is difficult to understand how undifferentiated tissue whose differentiation is held in check by the other

differentiated parts, could persist in a system such as Holmes postulated. If it has no function in the system and is not in symbiotic relation with other parts, why should it not disappear? If, on the other hand, symbiotic relations between it and other parts exist, it should, according to Holmes, differentiate into something. It is evident therefore that something besides undifferentiated tissue must take the place functionally of the part removed if replacement is to occur.

We can most readily gain an idea of what this something is by means of a concrete example. In *Planaria* and various other triclads, where even small pieces are capable of replacing all parts, we find that the reactions of such pieces, while differing in degree from those of the original animal, do not differ essentially in kind. After removal of the head, for example, the piece reacts in much the same manner as when the head was present, though more slowly and with less energy. In *Leptoplana*, on the other hand, where regeneration of a head does not occur after removal of the ganglia, the piece without ganglia is at once and clearly distinguishable from the animal with ganglia by the character of its reactions.

In *Planaria* then, and in the other forms where replacement of the head and ganglia are possible, the piece still retains in some degree the functional characteristics of a head-region. In removing the head we have not removed the only region possessing such characteristics, but only the region which possesses them in the highest degree of any part of the animal. In *Leptoplana* removal of the head and ganglia leaves no part which can supply functionally, even in slight degree, their place, and formation of a new head is impossible.

We must conclude that the localization of visible structural differentiation in an organism is not necessarily coextensive with the localization of functional processes or conditions characteristic of this region, but may be limited to the region of greatest energy of these processes or conditions. It is a well-recognized fact that the so-called functional structure of bone, tendon, etc., represents only the most frequent or most energetic functional conditions, and there is every reason to believe that similar relations exist between structure and function in many other cases. The case of *Planaria*

cited above is in fact a demonstration that functional processes may be less sharply localized than the structures which represent them. The anatomical structure known as the head in *Planaria* is not the only region where "head-reactions" are possible, but it does represent the region where they occur with greatest energy and frequency in the normal animal. Admitting this, the question arises as to why heads do not form all along the body in *Planaria*, i. e., as to why structure should be thus more narrowly localized than function. The answer is not far to seek. If two parts, one of which is capable of reacting in a certain manner more rapidly and with greater energy than the other are correlated, the reaction to a given stimulus will occur in the first part earlier and with greater energy than in the second. The fact that a reaction has occurred in the first part must bring about changes in the system in consequence of which the character of reaction in the second part is altered. If structure is, as I believe, the visible expression of functional or dynamic conditions, we cannot expect that the second part, even though it possesses in some degree the same functional capacities as the first should exhibit the same structure, for the very fact of its correlation with the first part which possesses these capacities in greater degree determines that the functional conditions in it shall be different from those in the first part. In general terms we may say that the region where a particular functional complex occurs with greatest energy, frequency or rapidity dominates so far as this particular complex is concerned all other parts of the organism which possess the same capacity in less degree, and modifies their activities to a greater or less extent. Consequently the structure with which a particular functional complex is associated in the normal animal may be much more narrowly localized than the functional complex. In *Planaria*, for example, the head-structure is limited to the anterior end of the animal, while the functional capacities commonly regarded as characteristic of the head exist at all levels of the body. These other regions are capable of producing a head-structure, *but only when isolated from the original head*.

It is evident then from this consideration that localization of visible structure is not necessarily an exact criterion of localization of

functional capacity. In all cases where a difference in localization exists, structure is more narrowly localized than functional capacity. On this fact, which I believe to be of fundamental importance for the problem of form, depends the ability of a part to become a whole when isolated.

In order to bring out clearly the difference between Holmes' hypothesis and my own, we may make use of Holmes' diagram (Fig. 1). According to my hypothesis, the various parts, *A*, *B*, *C*, *D*, etc., though perhaps visibly different as regards structure, each possess the physiological properties of the others or of some of the others in some degree. *B*, *G* and *F*, for example, the parts contiguous to *A*, are capable in some degree of activities similar to those characteristic of *A*, but as long as *A*, a region of greater energy or frequency or rapidity as regards these particular activities is present the correlations arising from it obscure, inhibit or modify the activities of *B*, *G*, *F*, so that they appear structurally and functionally to be different from *A*. But when *A* is removed, the parts *B*, *G*, *F* become at once the dominating parts as regards the *A*-activities and the correlations between them and other parts become similar in kind, to those which previously existed between *A* and the other parts, though probably different in degree. In short *B*, *G*, *F*, or certain portions of them are substituted functionally for *A* simply because in the absence of *A* their activities must, by virtue of their constitution, be somewhat similar to those of *A*. No entelechy or other peculiar principle is needed to guide or determine this substitution. It occurs with the same certainty as any other physical phenomenon in all cases where these parts possess the functional capacities to which attention has been called above. According to this hypothesis, the undifferentiated cells postulated by Holmes are not only unnecessary, but could not substitute for *A* if present, because the parts *B*, *G*, *F* are more like *A* than are the undifferentiated cells and would therefore dominate in the process of substitution.

The *A*-processes are undoubtedly in most cases, if not in all, at first less energetic or less rapid or both, than they originally were in *A*, and in consequence of this difference the system may regain its original condition of equilibrium in either one of two ways. If

the other parts *C, D, E* are plastic, i. e., if their activities are readily and rapidly altered by altered conditions, they will be affected by the decrease in *A*-correlations following the removal of *A* and will undergo more or less change in response to the changed correlations, i. e., regulation by what we ordinarily call redifferentiation will occur. If, on the other hand the parts *C, D, E* are relatively stable, i. e., not rapidly changed by altered conditions, they and the correlations arising from them will remain much the same as before the removal of *A*. But the *A*-processes in *B, G, F* are out of proportion to these correlations and must be quantitatively increased by them. In this case then equilibrium is regained by functional hypertrophy of the portions of *B, G, F*, which are the functional substitutes for *A*. This is what we know as regeneration in the stricter sense, i. e., formation of new tissue from the regions adjoining the cut surface and its visible differentiation with increase in size into a part like that removed. In most plants and in some animals regeneration occurs from regions more or less distant from the cut surface, simply because these regions are physiologically more like the missing part than is the region at the cut surface.

As a matter of fact, since correlations in the system are at least in large measure mutual, most if not all cases of restitution are mixtures of redifferentiation and regeneration. Some change, i. e., some redifferentiation occurs in some or in all parts of the system and some regeneration, i. e., functional hypertrophy of the part which forms the physiological substitute for the part removed takes place.

Holmes' hypothesis fails to recognize the fundamental fact, viz: that something must remain after the removal of a part, *A*, which can take its place functionally in the system in some degree. Without this the only factors which can prevent progressive departure from the original condition are lack of plasticity in the parts remaining or one-sided relations between parts. As a matter of fact however plasticity is a conspicuous feature in many forms in which the regulation of parts into wholes occurs most readily, and on the other hand all the evidence indicates that correlations are in large measure mutual rather than one-sided. In those cases

where a part after isolation is incapable of becoming a whole, while the remaining parts are capable of replacing it, there is reason for believing that the correlations are more or less one-sided, i. e., the part in question has been so greatly modified by the past or present correlations arising from other parts that it has lost its totipotence and can never become a whole, but the correlations arising from this part have not been sufficient to modify the other parts of the system to an equal extent. Examples under this head are the appendages of arthropods, amphibia, etc.

One other point discussed by Holmes requires brief consideration: in his first paper he selected the regulatory development of a head in *Planaria* as an illustration of the working of social pressure. In his discussion of this case differentiation is regarded as proceeding from the cut surface distally, in consequence of the social pressure exerted on the new parts by the old (Holmes '04, pp. 282, et seq.). In my criticism of this point (Child '06a, p. 421, et seq.), I called attention to the fact that in *Planaria*, and in other forms as well, differentiation of the regenerating tissue actually proceeds in the opposite direction, i. e., from the tip toward the base. In reply to my criticism Holmes ('07, pp. 427, 428) points out that the first visible differentiation is not necessarily the first actual differentiation, that "before any external features are produced in the development of a limb the main outlines of its differentiation may have been established through influence proceeding from its basal part, after which the tip might differentiate more rapidly than the intervening portion and the other visible features of structure appear successively toward the base." He also points out that in many cases the visible differentiation is centrifugal rather than centripetal and cites the case recently described by Zeleny ('07) of the antennule of *Mancasellus*, in which visible differentiation at first proceeds from the base toward the tip, but later in the opposite direction. He continues: "But granting that, in many cases, differentiation actually begins at the extremity and works toward the base of the regenerating organ, the process is not inconsistent with the point of view here set forth. We may suppose that the influence of the environment causes the extremity of an organ to begin to differentiate

like that of the missing part. That is only one step. We have then to account for the numerous coördinated differentiations that take place as the part develops toward the base. * * * The fact that, with few exceptions, such as the failure to regenerate the intermediate segments of the appendages, etc., the whole organ, nothing more nor less, is regenerated, and forms a congruent union with the basal part, is indicative of close interaction of the various parts of developing organs with the body of the organism at all stages of the process.

"I am inclined to think that neither centrifugal nor centripetal differentiation, expresses the entire truth of the matter, but that the new part differentiates as a whole, much as organs do in embryonic development, and at all times in intimate functional relations with the old part, differentiation becoming accelerated in one part or another, according to special conditions" (Holmes '07, pp. 428, 429).

As regards most of these points my position does not differ very widely from that of Holmes. My criticism of his analysis of the case of *Planaria* was directed primarily, not at his hypothesis in general but merely at his failure to consider the actual facts in that case. I see no reason why the occurrence of differentiation in either direction or in both should constitute a fatal objection to his hypothesis or to my own, for such differences are merely incidental and depend on the conditions in individual cases. When my criticism was written the experimental data seemed to indicate that visible differentiation in the centripetal direction was the general rule, though by no means without exceptions, and since Holmes did not in his first paper attempt to account for this fact in any way, his hypothesis was open to criticism. I certainly had no intention of maintaining that differentiation must in all cases proceed centripetally, since at that time various cases were known to me in which visible differentiation proceeded centrifugally.¹ I do not believe however that Holmes' suggestion that

¹ In his discussion of the direction of differentiation in the antennule of *Mancasellus*, Zeleny ('07, p. 335) says: "Child has recently expressed the opinion that differentiation must in every case proceed from the tip toward the base and in no other way." My actual statement was that differentiation from the tip toward the base is "a general rule in cases of regeneration." This statement as it stands is

the new part differentiates as a whole, much as organs do in embryonic development is universally applicable. There are certainly many cases in which the terminal portions attain or approach their final condition of functional activity before the basal parts are formed, and in a considerable number of cases also the basal parts are replaced incompletely or not at all. In fact it seems to me that such cases might be expected to occur, for in a correlated system the conditions for the regulatory formation of non-terminal regions must, at least sometimes, be largely dependent on the existence of typical functional conditions in terminal parts. If conditions in the terminal parts are more important than those in the old parts as determining factors in the differentiation of intermediate parts we should expect to find the intermediate parts differentiating later than the terminal parts, but if, on the other hand, conditions in the old parts are the chief determining factors, differentiation might occur wholly in the centrifugal direction.

Moreover, although I agree with Holmes that the absence of visible differentiation does not necessarily imply absence of physiological differentiation, I am incined to believe that the direction of progression of visible differentiation is not without significance as an indication of the direction of progression of physiological differentiation. In other words, while the absence of visible differentiation proves little or nothing with regard to physiological differentiation, its presence may prove something. I think it probable therefore that in some cases the regenerating part is not differentiated as a whole, but that its various regions are determined successively in one direction or the other: in other cases it may perhaps be differentiated as a whole. It would appear that none of these possibilities conflict with either Holmes' hypothesis or my own.

undoubtedly open to misinterpretation and should have been qualified, for I was well aware at the time it was made that centrifugal differentiation occurred in various cases. In fact, I had shown in earlier papers (e. g., Child, '04b) that the differentiation of the intestine in regenerating parts of *Leptoplana* is apparently centrifugal. However I take the present opportunity to make acknowledgments to Holmes and Zeleny for calling my attention to this misleading statement, and also to make clear my real position in the matter, which is that differentiation may occur in either direction or in both according to conditions in the particular case.

To sum up: Holmes and I agree in that we both postulate a condition of physiological equilibrium, or rather, as I should put it a condition of oscillation or cyclical change about equilibrium, as the basis of our hypotheses. The chief point of difference between us is that Holmes' hypothesis does not, as I understand it, provide for the maintenance of or return to the typical condition, except by the assumption of relations largely one-sided, or that of lack of plasticity. While these assumptions may serve for certain individual cases, they seem to me to be totally inadequate for the analysis of form-regulation in general. According to my own hypothesis a part can be replaced only when some other part is physiologically sufficiently similar to it to perform its functions at least qualitatively, if not quantitatively, after its removal.

The independent formulation of two hypotheses of form-regulation so similar in general point of view as are Holmes' and my own, is I believe not without significance, since agreement between different observers as regards the general nature of problems may be an indication that real progress in the analysis of data is being made. It is desirable in such cases, and particularly in fields where the data are so varied and complex, that differences of opinion should be fully and critically discussed. For this reason I have ventured to consider at some length in the present paper the points which seem to me debatable, and to state my own position in a manner which I hope will lessen the chances of future misunderstanding.

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THE PROCESS OF HEREDITY AS EXHIBITED BY THE DEVELOPMENT OF FUNDULUS HYBRIDS¹

BY

H. H. NEWMAN

WITH FIVE PLATES AND SIXTEEN FIGURES IN THE TEXT

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¹ Contributions from the Zoological Laboratory, University of Michigan (No. 116), and from the Woods Hole Marine Biological Laboratory.

I. INTRODUCTION

The experimental work on heredity of the last decade or so has, I believe, dealt too exclusively with definitive characters and has overlooked the origin and development of these characters. The usual method of procedure in such experiments is to interbreed two species or varieties exhibiting well marked differentiating characters, usually of a superficial sort, with the idea of determining for each character of the one species or variety whether it blends with or dominates the corresponding character of the other species or variety. The characters studied are nearly always definitive characters and can be observed only in advanced young or in adults.

Such work, it seems to me, overemphasizes the importance of one stage in the developmental process. That the definitive stage of a character is a comparatively fixed one does not obviate the necessity of studying the origin and ontogeny of such a character. But in the kind of work referred to above all other stages in the process, no matter how interesting and instructive, are overlooked. This tendency to regard the condition of characters in the adult as the whole of heredity has led to the publication of this paper as a plea for the study of heredity as a developmental process. In the present work it appears that sometimes the maternal and sometimes the paternal influence is uppermost. An individual or a strain may in early stages show a predominance of maternal characters and later a predominance of paternal, while in between the first and last stages may be seen an apparent struggle between the paternal and maternal forces, expressing itself in an alternating dominance of one or the other.

The work consists of observations of the comparative developmental histories of two pure breeds and their reciprocal crosses, the study being continued from fertilization until long after hatching. It makes no pretense of being a thorough-going study of Teleost embryology. Only such points in the developmental processes are noted as proved themselves efficient for the comparative study of rates of development, sizes of whole or of parts, degree and kind of pigmentation, functional activity,

physiological resistances, etc. Only to this extent then is the developmental history of the bony fish treated, hence no apology is offered for the incompleteness of embryological detail.

The experiments of Boveri, Driesch, Herbst, Seeliger, Fischel, etc., have for this work only a casual significance for the reason that the prime interest of the authors was focused upon another problem, viz: the comparative potency of nucleus and cytoplasm in heredity. No attention was paid to heredity as a process and this is the chief idea brought out in the present work. No formal review of this rather voluminous literature on Echinoderm hybrids is attempted in this place. Only where the facts presented have a distinct bearing on the work in hand will the data of these authors be referred to.²

Probably no type of egg offers so many advantages for the study of heredity as a process as that of the bony fish, and it is equally probable that few species of fish are so available for this kind of work as those used in the breeding experiments here described. Some of the most obvious advantages of the species used are as follows:

- 1 Both species are abundant and easily obtained.
- 2 Both species thrive well under laboratory conditions.
- 3 The adults are of convenient size, neither too large nor too small, and yield large numbers of eggs that are easily stripped.
- 4 The spawning behavior and sexual dimorphism of both species were easily studied and a knowledge of these phenomena proved to be a prerequisite for the present study.
- 5 The adults possess a sufficiently large number of differentiating characters to furnish data for the comparative study of adult hybrids, should it prove feasible to rear the young fish to maturity.
- 6 The eggs of both species are of convenient size, and, what is of more importance, are of quite unequal size.
- 7 The eggs are nearly transparent and the development of all characters can be studied without difficulty in living material.
- 8 The embryos can be studied for long periods as stationary

² For a comprehensive review of recent work on Echinoderm hybrids, see Alfred Fischel's paper, "Ueber Bastardierungsversuche bei Echinodermen." *Arch. f. Entw.-Mech.* 22, 1906.

objects within an envelope, needing no especial care and provided with their own food.

9 The egg membrane of both species is a thick, resistant capsule, capable of protecting the developing embryos from the various detrimental factors of their environment and making it more nearly possible to study heredity uninfluenced by extraneous phenomena.

10 Many other special advantages that apply only to the two species used will receive notice in the body of the paper.

II MATERIAL AND METHODS

Materials—A Descriptive Account of the Species Used

Fundulus majalis and *Fundulus heteroclitus*, two species of killifish belonging to the family Pœciliidæ, furnished the material for the breeding experiments detailed below. Both species abound along the Atlantic coast and are familiar to all workers at the Woods Hole Marine Biological Laboratories, where this work was done.

These two species interbreed readily and one of the reciprocal crosses is capable of hatching and of living in aquaria at least seven months after hatching. The two species present well marked differentiating characters in adults, embryos and eggs, both morphologically and physiologically. An account of these differences will furnish a logical introduction to the body of the work.

1. Morphological Differences Between the Adults

A reference to Plate I will serve to show the strikingly different general appearance of the two species. The actual average size of mature adults is represented in the figures and it will be readily seen that *F. majalis* is the larger though more slender species. A pronounced sexual dimorphism is exhibited in both species. This phenomenon is concerned chiefly with the shape and size of the fins, color pattern, degree of pigmentation, the presence in the males during the breeding season of minute "contact organs" that are of use in clasping the female, etc. Although this sexual

dimorphism would seem to be of no moment for the purposes of this paper, since I have not as yet been able to rear hybrids to maturity, yet a knowledge of this phenomenon is necessary as an aid to understanding the methods employed. Under a subsequent heading is given a brief treatment of the essential points about spawning behavior and sexual dimorphism.

2 Physiological Differences Between the Adults

a *F. heteroclitus* is markedly more resistant to adverse conditions, such as foul water, lack of oxygen, presence of carbon dioxide, etc., than is *F. majalis*.

b *F. heteroclitus* is therefore found in habitats unfit for *F. majalis*, such as brackish and foul ponds, etc.

c *F. heteroclitus* is much less readily affected by confinement in small aquaria and carries on its spawning in captivity with perfect freedom. *F. majalis*, on the other hand, is rather sullen in confinement and frequently refuses food for some time after capture. Spawning in aquaria is very rare with *F. majalis*.

d The flesh is harder and the muscles stronger in *F. majalis* than in *F. heteroclitus*.

e The heart-beat, and hence the general circulation, is markedly less rapid in *F. heteroclitus* than in *F. majalis*.

These and other differences between the adults that might be mentioned concern us much less directly than do differences between the eggs and developing embryos of the two species.

3 Morphological Differences Between the Eggs and Embryos

a The eggs of *F. majalis* are decidedly larger than those of *F. heteroclitus*, the average diameter of the former being 2.7 mm. and that of the latter 2 mm. A calculation shows that the volume of the average *F. majalis* egg is over twice that of the average *F. heteroclitus* egg.

b The eggs of *F. majalis* are of a decidedly yellowish color, while those of *F. heteroclitus* are almost colorless and more nearly transparent. These differences are due to a different composition of yolk and protoplasmic content.

c The capsule surrounding the egg of *F. heteroclitus* is, after exposure to water for some time, decidedly fibrous and sticky, causing the eggs to clump up in a very disagreeable fashion. The capsule of the *F. majalis* egg is, on the other hand, scarcely fibrous or sticky and the eggs seldom clump. On this account they are more easily handled than those of the other species.

d The size of the developing embryos and of young fish on hatching is in the two species in proportion to the comparative volume of the eggs, that of *F. majalis* being about twice that of *F. heteroclitus*.

e The color pattern of the young fish before and after hatching is quite different in the two species, as are also the size and structure and pigment content of the chromatophores.

4. Physiological Differences between the Eggs and the Developing Embryos

a The eggs and developing embryos of *F. majalis*, like the adults, are much less resistant to unfavorable environmental conditions than are those of *F. heteroclitus*.

b The eggs of *F. heteroclitus* reach the hatching period in about two weeks on the average, while those of *F. majalis* require nearly three weeks on the average. As a corollary to this *F. heteroclitus* is at all stages of development markedly in advance of *F. majalis*. The two species on hatching are at same stage of development.

c The body and yolk of *F. heteroclitus* embryos become heavily pigmented after about three days of growth, while in *F. majalis* only a very faint pigmentation occurs until after seven or eight days. The color on the bodies of newly hatched young is much paler in *F. majalis* than in *F. heteroclitus*.

d The heart-beat of *F. majalis* is, stage for stage, much more rapid than that of *F. heteroclitus*.

e A fair percentage of hybrids from *F. heteroclitus* eggs hatch spontaneously and are capable of living and thriving for months while none of the hybrids from *F. majalis* eggs ever hatch.

A number of other differentiating characters might be listed here, but it seems advisable to defer mention of many such characters until they can be treated in a connection more intelligible.

Method

Since the problem in hand has proven to be so largely one of method, it seems necessary to preface the bare statement of the method finally evolved with an historical account of some of the steps in the evolution of this method.

The first season's work brought out so much of contradiction and ill success in rearing both pure and hybrid strains, that it seemed necessary to become more familiar with the physiology and behavior of the two species of fish used. This study was carried on during the early part of the second season and resulted in the discovery of many interesting facts about the spawning behavior, the significance of the sexual dimorphism displayed, and the sure signs, morphological and physiological, of high sexual tone. Although a full account of these phenomena has been published,³ it will be convenient in this place to set down some of the facts that have a bearing on the present work.

1 Spawning Behavior and Sexual Dimorphism

These two species of fish, like other fish, have a well-defined breeding season. That of *F. majalis* is somewhat earlier than that of *F. heteroclitus* and lasts for a shorter time. In both species there is a still more restricted period during which spawning is carried on most actively, and during which both sexes are at the height of their sexual tone. This sexual climax comes earlier in *F. majalis* than in *F. heteroclitus*, by about two weeks, and overlaps the corresponding period of the latter by about three or four weeks.

During this period of a few weeks, which may be called the spawning period proper, both species show marked changes in structure and behavior. In the males an intensification of pigment appears over the entire body and especially in certain

³ H. H. Newman. Biol. Bull., vol. xii, no. 5, April, 1907.

regions. A sort of steely glint, somewhat akin to iridescence suffuses the body. On certain well-defined regions of the body appear many characteristic, somewhat stiff, papillæ that are readily visible to the naked eye. These papillæ are temporary organs of definite structure, that appear only during the spawning season and disappear afterward. They occur on dorsal and anal fins, on the cheeks, and on the sides, regions that come into most intimate contact with the body of the female during the act of spawning. These papillæ I have chosen to call "contact organs."

In the female the body becomes paler than usual, the flesh becomes soft, the fins soft and pliable, and the abdomen distended with eggs. They also show a coyness of behavior that seems to incite the males.

The spawning act proper, as observed both in aquaria and in the open, is essentially a clasping phenomenon. The male pursues and corners the female, crowds his body against hers and clasps her just anterior to the tail with his large, strong, and especially modified dorsal and anal fins. While united in this fashion, both bodies bent laterally into the shape of a flattened S, the tail regions vibrate rapidly for from one to three seconds and eggs and milt are simultaneously extruded in close proximity to one another, thus insuring fertilization. An account of courtship, rivalry and display, previous to spawning, while of some interest, would take us rather far afield at present. In a former paper these subjects are fully treated.

This study of spawning behavior and the physical signs of high sexual tone explained some of the earlier contradictions and ill success and served greatly to improve the methods used.

2 The Importance of Equalizing the Physiological Condition of the Parents

Experience showed what ordinary judgment should have suggested that, if one wishes to eliminate the factors of over- and under-ripeness, staleness, etc., of sexual products, it is necessary to cross-fertilize only during the period when both species are at their sexual prime. Only if this precaution be observed can one obtain in different experiments even approximately uniform results.

Not only must one be careful as to the time for starting experiments, but individuals must be carefully chosen. Males and females, full grown and sexually mature, as indicated by the sure signs of the sexual climax mentioned above, should always be used, for if eggs are stripped from females that are under-ripe only a small per cent of the eggs are capable of fertilization and the development of those is apt to stop short of completion. If males that have either failed to reach or have passed the sexual climax are used, their milt may be either entirely or largely ineffective in initiating normal development.

The eggs and milt of fish kept longer than three or four days in aquaria very frequently become stale, and very unsatisfactory results have been obtained from their use. These stale eggs are frequently capable of fertilization and of partial development, but the embryos usually die before hatching. This precaution applies especially to *F. majalis*, which very seldom spawns in captivity and hence females may carry eggs in the oviducts for weeks after they would normally be extruded. *F. heteroclitus* females, on the other hand, have a habit of ridding themselves of over-ripe eggs without the assistance of the males. Consequently stale eggs are seldom found in that species. Stale milt may be avoided in both species by selecting only the most highly colored males, with well developed contact organs. *F. majalis* males will not retain their dusky spawning coloration for more than a few days in captivity, so there is little danger of obtaining stale milt from that source if reasonable care is taken.

A multiplicity of parentage in a single batch of eggs must be avoided, as there is considerable variability in the eggs taken from different females and in the milt of different males according to their size, age, and degree of sexual maturity. Unless the eggs of one female are used for both pure and hybrid strains, and the milt of one male for both species of eggs in one series of experiments, an undue amount of variability ensues as the result of extraneous factors that needlessly complicate the issue and frustrate all attempts to study pure heredity.

3 The Importance of Equalizing the External Conditions of the Developing Embryos

After some experience it was found necessary to limit the number of eggs in one vessel and to keep these well separated. The tendency to clump up, exhibited by the eggs of *F. heteroclitus*, causes marked variations in time rate of development and ultimate success in hatching. If eggs are too numerous or too crowded in one vessel only a few succeed in hatching and many abnormal conditions are manifested. *F. majalis* embryos do not endure crowding so well as do those of *F. heteroclitus*, so, as a rule, only about half as many of the eggs of the former species are used in an experiment as those of the latter species.

Several methods were used for keeping the vessels well aerated. Running sea-water was used, but it was found that a rusty deposit collected on the surface of the eggs. This deposit seemed to interfere with development; probably interrupted the gaseous respiratory exchange. Aquaria containing *Ulva* were used in some of the experiments, but these did not prove very satisfactory on account of the fact that the eggs lay still too long and frequently became covered with *Saprolegnia* and other molds. The method that gave the best results was simple. Large, flat, covered bacteria dishes were used and the water was changed daily by pouring off the surface and filling up with fresh sea-water. In this way disease was kept at a minimum and there was always an abundance of well aerated water.

It is absolutely necessary to pick out all eggs that have not been fertilized as well as embryos that die from time to time, as these, if left in the vessels, will infect the healthy individuals.

4 The Attitude that Must be Taken Toward Variability

Another lesson taught by experience in dealing with large numbers of eggs and embryos is, that the high degree of variability in different strains and in different individuals of the same strain, must not be regarded as an insurmountable difficulty in an endeavor to arrive at definite results. Variability, here as elsewhere, is to be expected, and one must accept it as he finds it and must

learn to see the outlines of fixity and regularity through the haze of a confusing diversity of conditions.

No two strains develop at the same rate, but are retarded or accelerated by various conditions of temperature, oxygen content of the water, etc.; only when a pure and a hybrid strain are started simultaneously and are treated alike, can any basis of comparison be attained.

Even in pure strains, moreover, there is a considerable amount of variability; imperfect eggs are always present and give imperfect and unhealthy embryos. The great majority of eggs in pure strains, however, especially in the case of *F. heteroclitus*, develop at a practically uniform rate for the first week. There is always a considerable degree of variability in respect to the hatching period, some individuals hatching as much as four days later than others.

In hybrids of both kinds the factor of variability is a much more important one than in the case of the pure strains. Here the range of variability is enormously increased and it is a matter of considerable difficulty, especially in advanced stages, to decide on the average or representative condition when a hundred or more embryos are examined. Considerable practice, however, has made it possible, with some degree of personal satisfaction, to select a specimen that represents either the most prevalent condition or a judicious mean between extreme variants. In some cases, especially during the first four days of development, the selection of a representative condition offers no serious difficulties. It must be understood, then, that, in the description and figuring of such representative individuals in the succeeding pages, these have been arbitrarily selected by the writer and that his best judgment has been used in the selection. Where actual measurements or numerical determinations of structures or functional activities could be employed there was no difficulty in selecting the most prevalent or the average condition.

A fuller discussion of this factor of variability, especially as it applies to hybrids, follows the presentation of data.

5 Methods Proper

The methods of experiment and study were naturally the direct outcome of the experience outlined under the three previous heads. By taking the precaution to equalize, as far as possible, the physiological condition of the parents and the external conditions of the developing embryos, and, at the same time, allowing for the factor of variability, it was possible to get results of a somewhat regular and invariable nature.

The method of procedure that gave the best results was as follows: Fresh, egg-laden females of good size of both species were selected. The eggs of a *F. heteroclitus* female were then stripped into one finger-bowl, those of a *F. majalis* female into another. The eggs were then stirred up with the finger so that those first extruded and those last extruded might be evenly distributed. Then about half of the eggs in the two bowls were transferred to two other bowls. Two males, one of each species, at the height of their sexual tone, as indicated by their dark colors, the presence of contact organs, etc., were then chosen. The milt of the *F. majalis* male was stripped into a very little sea-water, stirred and poured partly on one lot of *F. heteroclitus* eggs and partly upon one lot of *F. majalis* eggs, being stirred up with the eggs in both cases. After washing the hands in fresh water, which certainly killed all adhering sperm from the *F. majalis* male, the *F. heteroclitus* male was used to fertilize the remaining two lots of eggs. After allowing the eggs in all four bowls to stand for about fifteen minutes with the small amount of water used in the fertilization process, the excess sperm was washed out with fresh sea-water and the eggs were transferred to large, covered bacteria dishes, containing about a liter of fresh sea-water. Usually from one to two hundred eggs were allowed to develop in each dish. The water in these dishes was partially drawn off and renewed nearly every day. Eggs were dissected apart whenever a tendency to clump up manifested itself. All dead eggs or embryos were removed as soon as noticed. The water always smelled sweet and fresh in cultures treated as described, and a very large percentages of embryos developed and hatched in the two pure strains, especially in those of *F. heteroclitus*.

For purposes of study lots of about fifty eggs were drawn off periodically with a large pipette into Syracuse watch glasses and examined with the low powers of the compound microscope. The methods of study and observation were many and involved the use of both living and preserved material. Comparisons of differences between the four strains of each series were made in various ways. In early stages actual counts of blastomeres were made, both in living and in fixed material. In somewhat later stages, when the blastodisc begins to spread out over the yolk, diameter measurements of large numbers of blastodiscs were made with the aid of the ocular micrometer. The latter instrument was used for measuring head and body diameters, and other dimensions of more advanced embryos.

Camera lucida drawings of living individuals, selected as good representatives of a strain, were made at selected intervals. These camera drawings were made at the level of the table and showed a magnification of 28 diameters. All drawings were made at the same magnification. Details in some of the drawings were filled in from fixed and stained material, put up at the time when the drawings were made.

The study of fixed material was of great assistance in confirming the observations made upon living material, and frequently added much new data.

Since no two series gave the same results, I have decided to present the data of one successful series in the form of an abbreviated pictorial table (Plates II, III and IV). Other series can be briefly compared or contrasted with this. Any one of five or six series might equally well have been chosen, but this one is fairly typical and shows perhaps a little more than any of the other series examined. Some facts, however, are better brought out by other series. No one series is complete for the reason that one can make observations only at intervals and important stages may be passed over between observations. The entire history, however, can readily be pieced together from a considerable number of series.

The four strains of the series selected are figured in tabular form, nine stages being figured during the first week of develop-

ment. It is difficult to study whole embryos within the egg membrane for a longer period, as they become too opaque and the fluids within the membrane are apt to become cloudy and to render outlines too vague to admit of camera drawings being made. It was necessary for purposes of drawing and observation to dissect out from the egg membranes both hybrid and pure bred *F. majalis* embryos.

If the specimens selected for drawing were active it was necessary to quiet them with choloretone before drawings could be made. It was frequently difficult to find a specimen that lay in a position suitable for drawing and much patience was required in order to find individuals that were at once typical and fortunately disposed for drawing.

For convenience in reference the four strains pictured in the table (Plates II to IV) are lettered *H*, *h*, *m*, and *M*, capital letters referring to pure strains and the corresponding small letters to the hybrids from the same species of egg. (*H*) refers to pure bred *F. heteroclitus*; (*h*) to the hybrid strain from the eggs of *F. heteroclitus* and the sperm of *F. majalis*; (*m*) to the hybrid from the eggs of *F. majalis* and the sperm of *F. heteroclitus*; and (*M*) to pure bred *F. majalis*.

In the main body of the paper certain abbreviations will be used without further explanation:

For the pure *F. heteroclitus* strains the terms: pure heteroclitus, *H* pure, or simply (*H*) will be used.

For the hybrid strains from *F. heteroclitus* eggs and *F. majalis* sperm, the terms: hybrid heteroclitus, *H* hybrid, or simply (*h*), will be used.

For the pure *F. majalis* strains the terms: pure majalis, *M* pure, or simply (*M*), will be used.

For the hybrid strains from the eggs of *F. majalis* and the sperm of *F. heteroclitus*, the terms: hybrid majalis, *M* hybrid, or simply (*m*), will be used.

III DESCRIPTION OF EXPERIMENTS AND PRESENTATION OF DATA

Data Derived from the Study of Living Material

1. Type Series (Series 1) (Plates II, III and IV)

This series was started at 2 p.m., on July 2, 1907. Fresh lots of fish of both species were brought in and the conditions for the experiment were practically ideal.

The stages of development earlier than those presented in the table, as well as those later than the seven day period (the last stage pictured in the table), will be described verbally or with the aid of occasional isolated illustrations.

Conditions earlier than Stage I (18 hours). a Comparative fertility of eggs to sperm of their own and that of the foreign species.

Out of 121 eggs of *H* pure, 108 cleaved (89 per cent fertile).

Out of 136 eggs of *H* hybrid, 84 cleaved (61 per cent fertile).

Out of 92 eggs of *M* pure, 82 cleaved (88 per cent fertile).

Out of 103 eggs of *M* hybrid, 57 cleaved (45 per cent fertile).

In both cases the eggs were more fertile to sperm of their own than to those of foreign species.

b Comparative rates of cleavage of pure and hybrid strains. After two hours nearly all of the *H* pures and the *H* hybrids had cleaved and were in the two-cell stage. About twenty minutes later over half of the eggs in these two strains were in the four-cell stage. Rapid counts of blastomeres of stages up to the sixteen-cell stage convinced me that there was no appreciable difference in the rates of early cleavage in pure and hybrid strains. Three hours after fertilization (one hour later than in the heteroclitus strains) both majalis strains had cleaved and were in the two-cell stage. They also showed equal rapidity in passing to the four, eight, sixteen-cell stages.

Other stages up to Stage I (18 hours) were passed over in the night. No observations were made during this period.

Stage I (18 hours) (see Plate II): Ocular micrometer measure-

ments of the blastodiscs of twenty eggs of each of the four strains gave the following figures:

- H* pure (*HI*): 16, 18, 16, 17, 17, 15, 18, 18, 16, 17, 17, 16, 15, 18, 18, 18, 17, 18, 17, 17. Average 17 + mm.
H hybrid (*hi*): 13, 13, 15, 14, 14, 15, 11, 17, 12, 16, 14, 13, 18, 12, 12, 13, 14, 15, 16, 14. Average 14 mm.
M hybrid (*mi*): 14, 14, 16, 17, 12, 12, 13, 18, 18, 17, 12, 11, 14, 14, 14, 15, 15, 16, 18, 18. Average 15 - mm.
M pure (*MI*): 15, 16, 15, 15, 16, 17, 14, 15, 15, 15, 15, 15, 15, 16, 15, 16, 16, 14, 13, 14. Average 15 + mm.

These measurements were made in less than half an hour in the order listed. The *H* hybrids had about fifteen minutes advantage in time over the *H* pures and yet show a lower average blastodisc diameter. A higher degree of variability is also noticeable at this early period. The measurements of the two *majalis* strains show no marked differences between them. The slightly greater average diameter of the *M* pures might be due to the time advantage of about fifteen minutes that elapsed between the measurements of the two strains. After determining the average diameters of blastodiscs in all four strains camera drawings of an average embryo of each strain were made and are reproduced in the table. The measurements made with the ocular micrometer, when compared with a micrometer scale on the stage, showed a magnification of 17 diameters, so that, in order to reduce the measurements to actual millimeters the figures must be divided by 17.

The difference in the average diameters of blastodiscs between *H* pure and *H* hybrid was so marked that it must have been evident for some time.

Stage 2 (24 hours) (Plate II): The majority of the *H* pure embryos showed the germ ring nearly halfway around the yolk and a fairly well defined embryonic shield. The condition seen in practically all of the specimens of *H* hybrids was distinctly less advanced than in the *H* pures, the blastodisc still forming a shallow cap over the yolk mass. No measurements were made of

this condition since it was sufficiently obvious without measurement (Plate II, *H2* and *h2*).

In *M* hybrids ocular micrometer measurements of the diameters of twenty blastodiscs were as follows: 21, 20, 23, 19, 21, 21, 19, 23, 18, 20, 20, 22, 21, 19, 17, 20, 21, 20, 22, 18. Average 20.25 mm. (Plate II, *m2*).

Twenty *M* pures gave 18, 16, 16, 17, 18, 16, 17, 17, 18, 18, 16, 17, 18, 17, 16, 18, 16, 18, 17. Average 17 mm. (Plate II, *M2*).

The *M* pure and *M* hybrid embryos seemed to be about eight hours behind the *H* pure and *H* hybrid embryos respectively. The *M* hybrids showed a distinct and measurable advantage over the *M* pures of the same age. According to measurements, then, the two pure strains show the extreme differences in time rate of development, while both of the reciprocal crosses show intermediate condition.

Stage 3 (48 hours): Nearly all of the *H* pures showed the condition figured in Plate II (*H3*). The optic vesicles were large and showed the cavity plainly. Three or four mesoblastic somites were visible on practically all of the specimens examined. Out of fifty-five specimens examined two were noticeably retarded, showing much smaller optic vesicles without a visible cavity, and no mesoblastic somites.

The *H* hybrids showed a wider range of variability. Of the forty-seven examined seven or eight presented a retarded condition similar to that of the two retarded specimens of *H* pure. Six showed a condition still less advanced, resembling (*m3*), but with the blastopore closed. No somites were visible on any of the specimens. On the whole it was an easy matter to determine that the *H* pures were considerably in advance of the *H* hybrids.

With regard to the *M* pures and the *M* hybrids it was a matter of considerable difficulty to determine which of the two strains was in the lead (Plate II, *m3* and *M3*). A fairly large per cent of the *M* hybrids, however, showed a condition more advanced than any *M* pures. One might be justified, then, in claiming that the hybrid strain was in advance of the pure. Other series, as will be seen, showed a much more marked difference between these two strains at the same stage of development.

Stage 4 (54 hours): The *H* pures showed a very uniform condition, like (*H*₄). The lens was well developed and the optic cup was invaginating. The optic vesicles were clearly defined. The primary interbrain was slightly lobed. There were on all healthy specimens examined about twelve somites. A few very much retarded specimens occur (Plate III, *H*₄).

The *H* hybrids were markedly behind the *H* pures. The most advanced condition resembled closely (*H*₃), but the majority were like (*h*₄). About 25 per cent resemble (*h*₃). The most advanced specimens had only four or five somites (Plate III, *h*₄).

The *M* hybrids showed very little difference from the *M* pures so far as the overgrowth of the germ ring and closure of the blastopore were concerned, but the optic vesicles of the former were considerably better developed than those of the latter. The condition of the average *M* hybrid was scarcely more advanced than (*h*₃), (Plate III, *m*₄).

M pures show a condition less advanced than *M* hybrids, between (*h*₃) and (*m*₃). *M* pures were from eight to ten hours behind *H* pures at this stage (Plate III, *M*₄).

Stage 5 (72 hours): The *H* pures had taken a very marked lead upon the *H* hybrids, for which the earlier establishment of a heart-beat, with the accompanying vitelline and body circulation, was probably responsible. The various brain lobes were large and well defined. The optic cup was fully invaginated and the lens spherical. The embryonic eye showed a considerable cavity between the lens and the inside of the optic cup. Accompanying the establishment of a circulation a large amount of pigment had been laid down in the form of dark brown chromatophores, scattered over the body and the yolk mass. On the latter they originate in connection with the capillaries. The trunk had become opaque with pigment. The embryos had, as a rule, quickened, wriggling of the tail region being very noticeable. The rates of heart-beat in ten specimens were as follows: 90, 98, 96, 92, 98, 97, 95, 98, 99, 95. Average, 95.9 beats per minute (Plate III, *H*₅).

The *H* hybrids (Plate III, *h*₅) were not nearly so advanced as the *H* pures, showing a condition only slightly more advanced than that shown by the *H* pures at fifty-four hours (*H*₄). Of

forty specimens examined four showed the feeble beginnings of a heart-beat, which was slow and irregular and at the rates of 26, 36, 44 and 42, an average of 37 beats per minute. These four specimens and some others were somewhat larger in size than the rest and showed a slight differentiation of the primary brain vesicles. The optic vesicles were well cupped and the lens spherical in all, but there was marked variation in the degree to which the heads of the various embryos had developed. The auditory vesicles were in all well defined. There were many somites, usually over twenty. In none of the specimens was there any pigmentation either on the body or on the yolk. In none had quickening occurred.

The *M* hybrids were at this time well in advance of the *M* pures. The average condition is figured in the table (*m5*). The head region of the majority of the *M* hybrids showed a condition even more advanced than that of the other hybrid strain (*h5*). There was a marked outlobing of the inter-brain. The optic vesicles were well cupped, the lens spherical and embedded in the cup. Auditory vesicles were well defined. There were fifteen somites, on the average (Plate II, *m5*).

The *M* pures were less advanced, showing a less well developed head region, and an average of about nine somites. No pigmentation present on either of the majalis strains (Plate III, *M5*).

Stage 6 (80 hours): During this rather short daylight period of six hours, from 2 to 8 p.m., on a warm day, a considerable change in the interrelationships of the four strains was apparent. The *H* pures had advanced slightly. The heart-beat had increased to an average of 110 beats per minute in ten specimens. The pigmentation was considerably heavier and the size of the embryos had increased noticeably (Plate III, *H6*).

In the *H* hybrids, however, a remarkably rapid change had occurred. The establishment of a heart-beat and a circulation, early in the afternoon, had caused a very rapid advance and at this time the *H* hybrids were nearly as well advanced as the *H* pures. A few specimens were quite as advanced as any of the *H* pures. The rates of heart-beat in ten examined were: 100, 96, 90, 88, 90, 98, 92, 99, 90, 80. Average 92 +. No pigmentation

was as yet apparent in any specimen, in marked contrast to the condition seen in the *H* pures where the pigmentation was readily visible to the naked eye. The head region of the average *H* hybrid was noticeably less advanced than that of the average *H* pure (Plate III, *h6*). In some few specimens the embryos were still as small and undeveloped as were the average *H* hybrids at 72 hours (*h5*). These retarded specimens numbered about 10 per cent of the whole.

The *M* hybrids were still in advance of the *M* pures, but the difference was not so marked. In both of the *majalis* strains the number of somites had increased to such an extent that it was difficult to count them. The average size of the *M* hybrids was a little greater than that of the *M* pures, but this difference was not well marked. The *M* hybrids had begun to lag in development and the *M* pures had almost overtaken them (Plate III, *m6*, and *M6*).

Stage 7 (96 hours): The *H* pures had advanced chiefly in the acquisition of a more definite body outline. Pigmentation had become heavier and had rendered the body rather opaque, making it difficult to see the outlines of the brain and nervous system. The optic cavities were more pronounced and the eye had become slightly pigmented (Plate IV, *H7*).

The *H* hybrids (Plate IV, *h7*) had grown to be a little larger on the average than the *H* pures, but were comparatively pale, only a very faint pigmentation being visible. This failure to pigment at an earlier stage may be due to the fact that the paternal species does not show any pigment until several days later than this period. The heart-beats of ten numbered as follows: 126, 120, 118, 68, 112, 124, 120, 110, 128, 116. Average 114.2 beats per minute. This average is about seven beats to the minute faster than the average of ten *H* pures of the same age. The rates of heart-beat of all but one retarded specimen, with a rate of 68, are considerably faster than those of the latter. The specimens with beats of 126, 120, 124, 120, 128, were especially large and were noticeably more heavily pigmented than the others, although none of them were nearly so dark as the average *H* pure.

The *M* hybrids, in general body form, seemed to be about on an

equality with the *M* pures. Heart rates of ten gave the following: 84, 82, 94, 83, 90, 82, 86, 66, 90, 78. Average 83.5. The average state of advancement was about equal to that of *H* pures at 72 hours (*H*₅), (Plate IV, *m*₇).

The *M* pures (Plate IV, *M*₇) appeared very like the *M* hybrids. The heart rate, however, was very slow and feeble as yet, averaging 42 in ten specimens. Evidently the heart rhythm in *M* hybrids was established considerably earlier than in *M* pures. All embryos of both majalis strains were at this time entirely devoid of pigment. To the naked eye they appear very pale as compared with the heteroclitus embryos.

Stage 8 (114 hours): The past eighteen hours showed a marked advance in the *H* pure embryos. They had rounded out and now exhibited a typically fishlike form, with caudal fin well defined, the whole body opaque, and the eyes pigmented. The figure (*H*₈) will show the general appearance better than a verbal description. Large chromatophores were present on the head directly over the brain and upon the surface of the eye. The yolk mass was shrunken to a noticeable extent (Plate IV, *H*₈).

The *H* hybrids (Plate IV, *h*₈) were on the average not so advanced as the *H* pures, exhibiting a condition more like that of the *H* pures at 96 hours (*H*₇), except that many of the *H* hybrids were larger than the latter. The pigmentation was as yet considerably lighter than in the pure strain. The heart rate of ten *H* hybrids averaged 128 as compared with 112 for the *H* pures of the same age. More retarded and ill-developed specimens were noticeable than at any previous stage of development, probably on account of the failure of many to establish a normal circulation. A number of such ill-developed embryos were no farther advanced than the average *H* pure at 72 hours (*H*₅). On the other hand there were a few rather precocious specimens that were nearly as advanced as the average *H* pure of the corresponding stage (*H*₈).

The majority of the *M* hybrids (Plate IV, *m*₈) were in size and general structure about like the *M* pures. In certain details, however, they were very different. The majority were lightly pigmented both on the body and the yolk. The heart rates of ten

were as follows: 118, 112, 112, 104, 110, 110, 120, 110, 114, 98. Average 110.8. A few poorly developed specimens were noticed, resembling the condition of *M* hybrids at 80 hours (*m6*). About 90 per cent, however, were large and healthy looking.

The *M* pures were about the size of the best of the *M* hybrids. About 10 per cent, however, had died since the last examination. There were several ill-developed and anæmic specimens. The heart rates of ten specimens were as follows: 82, 80, 78, 78, 80, 82, 80, 80, 82, 82. Average 80.4. Although there was a complete vitelline circulation there was no pigment on any of the specimens. The heart-beat in the *M* pures was nearly two days later in appearing than in the *H* pures. The pigmentation was still more markedly slower in making its appearance (Plate IV, *M8*).

Stage 9 (168 hours or 7 days): Several stages were examined between the last stage described and the present one, but there was no very noteworthy change in the relative conditions of the four strains. At this period the *H* pure embryos had grown considerably in size, the eyes had reached almost their definitive form and were darkly pigmented. The pigmented areas were well marked and consisted of large chromatophores. The pectoral fins had appeared and were in a continual state of vibration, probably performing a respiratory function. The heart-beats were difficult to count on account of the opacity of the body, but from special studies on other series it appears that the rhythm established at Stage 8 is maintained at least up to hatching. The capillaries and vitelline vessels were covered with brownish black pigment. Larger chromatophores of a lighter brown color were found between the capillaries.

The *H* hybrids (Plate IV, *hg*) were on the average equal in size to the *H* pures (Plate IV, *Hg*), but as a rule were much less heavily pigmented, many being decidedly pale. About 10 per cent of the hybrid embryos were, however, at least as heavily pigmented as any of the *H* pures, and some of these dark hybrids were noticeably larger than the largest of the *H* pures. The poorly developed specimens showed all degrees of advancement. Some of them lacked a circulation, although the heart was present and was beating at a fairly high rate. The hearts of some were shrunken

and bloodless, mere strands that continued to contract rhythmically within an enormous empty pericardium. A number of these abnormal conditions will be described and discussed in a later section of this paper. The hybrids, although a heterogeneous lot, still showed about 60 per cent of normal, healthy specimens.

The *M* hybrids were too obscure within the envelope to admit of accurate camera drawings. A typical specimen, however, was dissected out of the envelope and, after being quieted with chloretone, was drawn (Plate IV, *m9*). The general body form is similar, except that it is larger, to that of the average *H* pure of 114 hours (*H8*). The caudal fin was well developed and the eye had assumed a form almost definitive. Heavy blackish pigment, in the form of large chromatophores, had darkened the surface of the yolk sac, while slender grayish, much branched chromatophores were distributed over the surface of the body.

The *M* pures (Plate IV, *M9*) were on the average about the same in size as the *M* hybrids. There was a small amount of blackish pigment on the yolk, but none at all on the body.

The verbal description of the stages figured in the table ends here. The further description of the series will be carried on without numbering the stages. The sections, instead of being numbered by stages will be headed with the number of days that have elapsed since fertilization.

At 10 days: The general size of the *H* pure and *H* hybrid embryos was about the same, the chief difference between the two strains being that the healthy *H* hybrids were a little more heavily pigmented than the average *H* pures. A few *H* hybrids were especially dark. The *M* hybrids showed a heavy pigmentation on the top of the head and down the middle of the back. The vitelline vessels were also very heavily pigmented, about like that of the average *H* pure at 96 hours (*H7*). The general size of the *M* hybrids was less than that of the *M* pures. There had appeared a change within the last two days in the *M* hybrids. They seemed to have lagged behind the *M* pures after having been at first ahead of the latter and, for some time past, practically on an equality with them. The *M* pures although

now noticeably larger than the *M* hybrids, were not nearly so heavily pigmented. A certain degree of pigmentation had appeared since the last stage described, but the color was still hardly visible to the naked eye. The chromatophores of *M* pure were small-bodied and much branched, quite different in appearance from those seen on the *H* pures and the two types of hybrids. The chromatophores of these three strains are practically alike. They are much thicker bodied and less branched than those of *M* pure. In other words, the type of chromatophore of *F. heteroclitus* seems to be dominant.

At 11 days: The *H* hybrid embryos, with the exception of about 25 per cent which were ill-developed and obviously unhealthy, showed heavier pigmentation than the average *H* pure. The chief reason for this seemed to be that the pigment of the paternal species (*F. majalis*) is blacker, even if more diffuse, and, when aggregated in denser masses as it is in the hybrid, it gives a darker coloration. The *H* hybrid embryos were on the average larger than the *H* pures and the egg membranes seemed to be under some tension. The *M* hybrids seemed to have stopped growing, while the *M* pures had advanced rapidly. There was at that time a marked disparity in size between the *M* pure and *M* hybrid strains, the former being about 50 per cent larger than the latter. A few unhealthy *M* pure embryos were noticed at this time.

At 12 days: 24 *H* pures hatched out during the forenoon of this day, before any change in the water had been made, and hence without any artificial stimulus. A camera drawing of one of the best of these was made on hatching, the specimen being quieted with chloretone (Plate V, Fig. *H*). The figure shows a dorsal view. During the first four hours of the afternoon twenty-five more *H* pures hatched, along with two specimens of *H* hybrid. In almost every particular these two hybrids resembled the *H* pures that had just hatched. They were as darkly pigmented as the darkest specimens of the *H* pures, were a little larger than the average *H* pures, but not noticeably so. The color pattern on the head and back was identical with that on the *H* pures. These two hybrids were also as early to hatch as the average *H* pures, and hatched without any artificial stimulus. The *M* hybrids

had evidently ceased to develop. The largest specimens had reached a size almost equal to that of the average *H* pure on hatching. The color pattern of the head and back was almost identical with that of the two heteroclitus strains on hatching. The yolk was very heavily pigmented and was still a large mass. The *M* pures were on the average nearly twice as large as the *M* hybrids. The body was pigmented with large grayish chromatophores. The yolk was lightly pigmented with black.

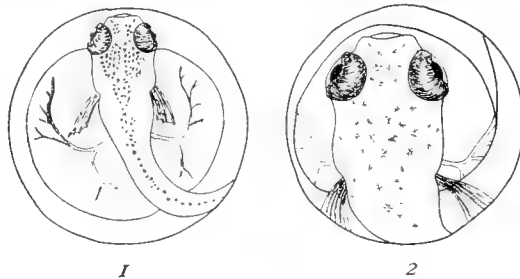
At 13 days: About fifty more *H* pures hatched. No more *H* hybrids hatched in spite of the water being changed and the consequent stimulus afforded by the mechanical disturbance and the sudden change of temperature. *M* hybrids and *M* pures in relatively the same condition as on the previous day.

At 14 days: Practically all of the *H* pures hatched. Only a few anæmic specimens left unhatched. No more *H* hybrids hatched, but they had become more heavily pigmented, especially on the yolk. A large specimen, with heavily pigmented yolk, was dissected out of the egg membrane and it lay almost inert in the sea-water, showing that it was not ready for hatching. The length of this specimen was 7.5 mm., while that of the first *H* hybrid hatched was 7.2 mm., and that of the first hatched *H* pure was 7.3 mm. This embryo then although not nearly ready to hatch, was somewhat longer than the hatched embryos of either *F.* heteroclitus strain. Late in the evening of the same day six more *H* hybrids hatched without artificial disturbance. *M* pures greatly increased in size, but *M* hybrids no larger than at the last observation.

At 15 days: Fifteen more *H* hybrids hatched. A typical specimen of this lot was quieted and drawn (Plate V, Fig. *h.*)

At 16 days: Twenty-two more *H* hybrids hatched. Only the abnormal specimens, that could never hatch, were left within the membranes. These latter number about 25 per cent of the whole. The *H* hybrids hatched out during the day were not nearly so heavily pigmented as those that hatched out earlier. They were also somewhat sluggish on hatching, some of them appearing to be cramped by over-long confinement within the egg membrane. A considerable number of those that hatched on this day did so

only on the application of some stimulus, such as squirting them violently out of a pipette or pricking the egg membrane with a sharp needle. Such specimens lack the initiative to hatch without some assistance and would have died within the egg membrane had not assistance of some sort been given them. When released these individuals swim about rather sluggishly, and, on being transferred to aquaria are very apt to float on the surface and allow themselves to be drawn by the currents to the gauze around the standpipe, and thus perish. Active young fish, such as the previously hatched *H* hybrids, always go to the bottom and, if drawn by a current, swim actively away from the region of danger.



Figs. 1 and 2 show comparative sizes of *M* pure and *M* hybrid embryos at nineteen days (Series I).

Fig. 1 represents an average specimen of *M* hybrid.

Fig. 2 represents an average specimen of *M* pure.

The figures are camera drawings of embryos as they lay within the egg membrane, quieted with chloretone. Figures show a magnification of 12 diameters.

At 19 days: No more *H* hybrids hatched, but many still living. The *M* pures had grown considerably and looked as though they were almost ready to hatch. A fairly heavy deposit of grayish pigment covered the body but was sparingly scattered on the yolk mass. The *M* hybrids showed no change except that they had grown darker. Outline camera drawings of typical embryos of the two majalis strains at this stage will emphasize the very marked difference between them (Figs. 1 and 2).

At 22 days: About twenty *M* pures hatched. A typical specimen, drawn on hatching, is shown in Plate V, Fig. *M*. This specimen was 11 mm. in length as compared with 7.2 mm. for the *H*

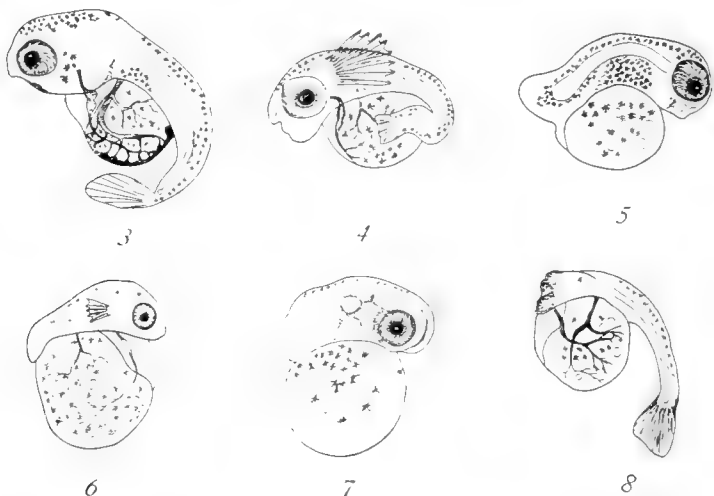
pure drawn on hatching. The head diameter of this *M* pure was 1.8 mm., as compared with 1.3 mm. for the *H* pure. Two more *H* hybrids had hatched since the last examination, but were dead when noticed. Both specimens were pale, twisted and emaciated.

At 23 days: About thirty more *M* pures hatched. Nearly all of the rest were dead, many of them having reached almost the maximum size before dying.

At 27 days: All embryos of the series except about thirty *H* hybrids and about sixty *M* hybrids, were dead. The *H* hybrids showed a wider range of developmental stages and abnormal conditions than the *M* hybrids. The latter were a fairly healthy looking lot, seemingly quite normal except for the presence of the large yolk mass attached to them. Some of them, when liberated by dissecting off the egg membrane, swam about quite actively and continued to live for several days in sea-water, although handicapped by the presence of their abnormally large yolk sacs full of a kind of material that they seem utterly unable to assimilate. I tried to free them from their encumbrance by pricking the sac and liberating the excess yolk, but the specimens thus operated soon died, probably from loss of blood, since some blood was seen to escape when the yolk material issued from the sac. A number of the *M* hybrid embryos, liberated artificially from the egg membrane, lived for as long as four days, but during this time the yolk mass did not diminish and the embryos probably starved. A typical specimen of these artificially liberated embryos was quieted and drawn (Plate V, Fig. *m*).

All of the *H* hybrids were dissected out of their membranes and presented a bizarre collection of freaks. A number of the types shown were drawn and will be seen in Figs. 3 to 8 inclusive. Fig. 3 presents the type most nearly normal. Fig. 4 is an unusual type, showing an enormous growth of pectoral fins, which seem to have continued to grow although the rest of the body had ceased to increase in size. This might be explained by the fact that the only noticeable circulation in the whole specimen was in these fins. Figs. 5, 6 and 7 show various degrees of a common type, in which the trunk region was greatly reduced. Fig. 8 shows a rare type, in which the head region was ill developed, although the trunk was

almost normal. In none of the specimens examined was the circulation normal. In some it was partial, while in the majority it was totally lacking, except in the heart itself. Much of the ill success in development was probably attributable to some lesion in the circulation, for which a reason will be suggested in the discussion.



Figs. 3 to 8 (inclusive) represent types of monstrosities in the *H* hybrid strain, dissected out of the egg membrane, after all specimens capable of hatching had done so. These are camera lucida drawings, showing a magnification of 12 diameters.

Fig. 3 represents a common type, well advanced, but with some lesion in the circulatory system.

Fig. 4 represents an unusual type, in which the pectoral fins and the eye have continued to develop independently of the cessation of growth in the rest of the body.

Figs. 5, 6 and 7 show types of the tailless monster, the most frequent abnormality.

Fig. 8 represents an uncommon type, a headless monster. The head region is represented by a very small, shrunken, heavily pigmented mass.

Considerable independence was seen in the development of different regions. Head may develop while trunk is retarded and vice versa. The fins in one case had continued to develop after the rest of the body had ceased to grow.

A considerable number of *H* hybrids of this series are at the present time being reared in aquaria at the United States Bureau of Fisheries at Woods Hole, Mass.

The data of five other series, almost as complete as the one just described in detail, are on hand and furnish many interesting comparisons and some new facts. There was a surprising uniformity of results in the three other series conducted at about the same time as the type series. After the development of a method the results of all experiments that were carefully performed were as similar as could be expected. Some of the earlier series, however, gave results that varied widely in some particulars from the type described. The plan followed is to take up first the series in which all precautions were used, presenting any new or interesting data as briefly as possible. Then will follow a very brief statement of the results of earlier series in which no special effort was made to equalize conditions of parent or embryos. Following that will come a statement of confirmatory or contradictory results of incomplete or partial series.

2 Other Series

Series 2, July 5, 1907. This was more like the type series than was any other complete series. The experiment was, however, conducted in a somewhat different manner. Fresh lots of fish were used and after three hours twenty eggs that had successfully cleaved were selected from each of the four strains. No careful counts of blastomeres were made but it was noted that there was no distinct difference in time rate of cleavage between the pure and hybrid strains.

At 14 hours: Ocular micrometer measurements of blastodisc diameters of all embryos, gave the following averages: *H* pure, .88 mm.; *H* hybrid, .87 mm.; *M* hybrid, .91 mm.; *M* pure .89 mm. The differences were too small to be significant and it is probable that the influence of foreign sperm had not made itself felt to a measurable extent as yet.

At 19 hours: Average blastodisc diameters of all eggs: *H* pure, 1.27 mm.; *H* hybrid, 1.12 mm.; *M* hybrid, 1.08 mm.; *M* pure, 1.11 mm. Here the difference between the *H* hybrids and the *H* pures was quite marked, but that between the two majalis strains was still very slight.

At 22 hours: Camera drawings of each of the embryos were

made and afterward measured in order to see what proportion of the yolk had been surrounded by the germ band. The results:

- H* pures, 24.6 per cent of yolk covered;
- H* hybrids, 20.8 per cent of yolk covered;
- M* hybrids, 18.2 per cent of yolk covered;
- M* pures, 16.2 per cent of yolk covered.

At 30 hours: An interesting condition between Stages 2 and 3 of Series 1. Here the *H* pures had the blastopore almost closed and the optic lobes well developed, resembling (*h*₃). The *H* hybrids showed the germ band about three-fourths around the yolk and the embryo in the primitive streak condition, slightly more advanced than (*M*₃). *M* hybrids with germ band about one-half around the yolk and a germinal shield better developed than in *M* pures. Germ band in *M* pures less than one-half around the yolk.

The rest of the series offered only a few noteworthy differences to the conditions seen in Series 1.

A tabulated account of these differences follows:

a At 56 hours there was no marked difference in degree of advancement between *M* pures and *M* hybrid, in both the condition resembling closely (*M*₄).

b At 70 hours, however, the *M* hybrids showed a marked advantage over the *M* pures, the former resembling (*m*₅) and the latter (*M*₅).

c At the end of a week the *M* pures and *M* hybrids were decidedly clearer within the egg membrane and admitted of camera drawings. The drawings show the two strains to have been practically identical in size, but the *M* hybrids were heavily pigmented both on body and yolk, while the *M* pures showed no pigment on the body and only a few finely branched, grayish chromatophores on the yolk.

d The *H* pures hatched as follows: 2 on eleventh day, 14 on twelfth day, 2 on thirteenth day. The *H* hybrids hatched as follows: 1 on eleventh day, 10 on fourteenth day, 4 on fifteenth day, and 1 on sixteenth day. The remaining four showed decidedly abnormal conditions like those shown in Figs. 3 to 8, and never

hatched. The first 11 *H* hybrids to hatch looked darker and larger than the hatched *H* pures. The *M* pures hatched as follows: 4 on twentieth day, 6 on twenty-first day, 4 on twenty-second day; the rest died. None of the *M* hybrids hatched. Eighteen out of twenty of them were living on the twenty-fifth day and micrometer measurements of head diameters were made for the sake of comparison with those of the other strain on hatching. The following were the measurements 1.28, 1.24, 1.24, 1.14, 1.45, 1.24, 1.16, 1.36, 1.14, 1.1, .61, 1.28, 1.4, 1.36, 1.12, .82, .97, 1.24. Average 1.06 + mm. Average head diameters of *M* pures on hatching was 2 mm.; that of *H* pures, 1.24 mm. Except for the presence of three dwarfed specimens the average of the *M* hybrids would have been a little greater than that of the *H* pures and the specimen with head diameter of 1.45 mm. was larger than any of the *H* pures. Even this large specimen, however, was not as large as the smallest of the *M* pures measured.

Series 3, July 3, 1906. In this series no micrometer measurements were made, but as only ten embryos were followed for each strain, it was possible to become personally acquainted with nearly all of the specimens and judgment was based on this acquaintance.

At 25 hours: *H* pure, germ band one-half around yolk. *H* hybrid germ band one-third around yolk. No difference between majalis strains.

At 48 hours: *H* pure, between (*H*₃) and (*H*₄). Average somites, 8.

H hybrid, slightly less advanced than (*H*₃). No somites.

M hybrid slightly more advanced than (*m*₄).

M pure, slightly more advanced than (*M*₄).

At 62 hours: No very marked difference between *H* pures and *H* hybrids. *M* pures and hybrids also almost on an equality. At this time there seemed to be a halting on the part of the two strains that had been ahead, giving an opportunity for those that had been distinctly behind, temporarily to overtake them.

At 68 hours: Six out of ten *H* pures had slow and feeble heart-beats. No heart-beats in any of the other strains.

At 76 hours: In size and general body form the *H* pures were

like (*H*₅), *H* hybrids like (*h*₆), *M* hybrids between (*m*₆) and (*m*₇), and *M* pures between (*m*₅) and (*m*₆). *H* pure highly pigmented and with the following heart rates: 58, 58, 74, 62, 48, 70, 42, 75, 70, 56. Average 61.3. *H* hybrids no pigment and with the following heart rates: 74, 42, 66, 48, 76, 48, 64, 60, 108, 72. Average 64.8. *M* hybrids no pigment, following heart rates: 60, 48, 62, 60, 60, 52, 60, 56, 64 (one not yet beating). Average, exclusive of the last, 59. *M* pure no pigment and no heart-beat.

At 84 hours: Heart rates as follows:

H pure—78, 78, 84, 56, 78, 72, 72, 74, 80, 82. Average 75.4.
H hybrid—84, 74, 74, 80, 90, 78, 78, 94, 92, 94. Average 83.8.
M hybrid—88, 80, 96, 84, 86, 92, 90, 88, 84, 86. Average 87.4.
M pure—72, 62, 66, 66, 64, 60, 68, 72, 64, 60. Average 65.4.

At 108 hours: *H* hybrids seemed somewhat larger than *H* pures but were much more lightly pigmented. Heart rates as follows:

H pure—100, 108, 112, 100, 104, 112, 110, 102, 108, 100. Average 105.6.
H hybrid—116, 116, 140, 116, 112, 140, 142, 132, 152, 136. Average 130.
M hybrid—120, 128, 120, 124, 124, 124, 36, 140, 136, 128. Average 118.
M pure—132, 128, 132, 140, 144, 126, 132, 124, 140, 134. Average 133.2.

Other stages up to hatching not markedly different from Series 1. *H* pures hatched out on the average three days earlier than *H* hybrids. *M* hybrids did not hatch at all, but reached almost a uniform size, about equal to that of *H* pure on hatching. All *M* pures hatched in an average of about twenty-two days.

Series 4, June 23, 1907. This series was started with unusual care and was followed for five days under close observation. It

then became necessary to be absent from the laboratory for a few days. After returning the experiment was not followed in detail, but what records were taken show a striking resemblance to conditions seen in Series 1. The figures made with camera lucida during the first five days might almost be substituted, stage for stage, for the figures in the table used for Series 1.

Series 5, June 19, 1906. Fresh adults of both species, at the height of spawning season, used. Over 100 specimens of each of the four strains.

At 52 hours: *H* pure lens formed and many somites, resembling (*H*₄). *H* hybrid no lens and few somites, slightly more advanced than (*h*₄). *M* hybrids blastopore nearly closed and optic lobes beginning to appear. *M* pure blastopore still wide open and no optic lobes.

At 72 hours: *H* pures somewhat more advanced than (*H*₅). Pigment on body and yolk and hearts beating strongly. *H* hybrid about like (*m*₆). A few have very slow and feeble heart-beat. No pigment. *M* hybrid between (*H*₃) and (*H*₄). Lenses and optic cups just forming. Auditory vesicles faintly defined. No heart-beat and no pigment. *M* pures less advanced than *M* hybrid about like (*H*₃), scarcely any trace of lens formation, no auditory vesicles, and no pigment.

At 96 hours: *H* pures heavily pigmented. Hearts vigorously beating. Embryos wriggling within the egg membrane. *H* hybrids weakly pigmented in a few specimens. Heart-beat on the average more vigorous and rapid than in *H* pures. *M* hybrids heart-beat vigorous; no pigment; embryo wriggling. *M* pures, heart-beats beginning to be established in a few specimens; no pigment and no wriggling.

At 5 days: *H* pures very deeply pigmented with dark brown chromatophores; average heart rate of ten, 109.7. *H* hybrid not nearly so heavily pigmented as *H* pure; heart rate of ten, 113. *M* hybrid as heavily pigmented as *H* hybrid; average heart rate of ten, 109. *M* pure, no pigment on body; extremely faint pigment on yolk; average heart rate of ten, 99.

Other stages up to hatching were substantially like Series 1, except that the series as a whole developed more slowly; the *H*

pures taking an average of eighteen days to hatch, the *H* hybrids an average of twenty days, *M* pures an average of twenty-six days. No *M* hybrids hatched.

The survivors of the three strains that hatched were placed in balanced aquaria, were fed on clam juice, and generally carefully looked after. The *H* hybrids thrive in this environment and, on the average, outgrew the *H* pures. About a dozen of these *H* hybrids continued to live in the aquaria after all of the *H* and *M* pures had died. The survivors were transferred to an aquarium provided with a standpipe, so that they could have a continual flow of fresh sea-water, with the abundant supply of minute organisms that are found in it. The young fish grew rapidly on this diet and lived for seven months. During this time they were looked after by Dr. Sumner, to whom I take this opportunity of expressing my thanks for his kindness. Only four of these young fish survived until March, at which time they simultaneously sickened and died. A comparison of these young fish with pure bred specimens of about the same age and size reveals an interesting resemblance to both parent species. There is so little material on hand, however, that it is deemed wise to reserve a treatment of the advanced conditions until a greater degree of success in rearing the young has been attained. It seems in no way impossible to rear the hybrids to maturity.

Series 6, June 19, 1906. This series was started on the afternoon of the same day as the last series. Different parents were used and only ten specimens of each strain (selected after cleavage had begun) were followed. In all important details this series agreed so closely with Series 5, that it seems superfluous to record the data. It was, in fact, the striking resemblance between these two series reared under the same conditions, that suggested the idea of controlling future attempts at equalizing the developmental conditions of the embryos by equalizing the physiological conditions of the adults.

The six series above described are all that were studied in detail through the whole or nearly the whole process up to the period of hatching. Many other series, however, were started for other purposes; to furnish material for experimentation and preserva-

tion. Other series were conducted solely for the purpose of furnishing quantities of young fish for rearing. Still others were followed for a while and then abandoned for lack of time to record data, since it seems impossible to follow carefully more than about three series at one time. Still other experiments gave results out of harmony with the general trend of data. In fairness such series should at least be mentioned, although it seems quite certain that the contradictory results were the outcome of poor method. To distinguish these series from the more complete ones detailed above, the various series will be designated by letter instead of by number.

3 Fragmentary Data

Series A, June 20, 1906. Conditions of experiment not recorded. Like Series 1 except that at 18 hours the *H* hybrids were noted and drawn as being slightly more advanced than *H* pures. At 48 hours, however, the relative degrees of advancement shown in Series 1 had been attained. There seems to have been a retarding effect due to foreign spermatozoa, the same in kind but differing markedly in degree from that seen in other series. No further observations.

Series B, June 28, 1906. This was one of several series that served to show the importance of using freshly taken fish. In this case the fish used had been in aquaria for at least ten days and had not been regularly fed. Only one observation was made—at 24 hours—when the following data were obtained: About 50 per cent of *H* pures developing, the blastodiscs very small. Only 10 per cent of *H* hybrids developing, but these with blastodiscs more advanced than the *H* pures. About 50 per cent of *M* hybrids developing. Only about 5 per cent of *M* pures developing normally. The great majority of the blastodiscs were irregular, cleft or ruptured. None of this strain developed to an advanced condition. A few *M* hybrids reached a condition about like (*m8*).

Series C, July 8, 1906. An incomplete series, not especially contradictory. At 24 hours there was a much more marked difference between *H* pure and *H* hybrid strains than shown in Series 1. The blastodisc of the *H* hybrids was about in the con-

dition of (*H*₁) while the germ band of *H* pures was on the average more than halfway around the yolk. At 43 hours the blastopore in *H* pures was nearly closed, more advanced than (*m*₄), while in *H* hybrid the germ band was less than one-half way around the yolk and the embryo was very ill defined, between (*H*₂) and (*m*₃), nearer the former. At 89 hours *H* pures resembled (*m*₇); *H* hybrid (*m*₅); *M* hybrid (*m*₅); and *M* pures (*m*₆).

Series D, July 2, 1906. This series was reared to hatch and was observed only incidentally while the water was changed from time to time. Just before hatching, however, counts of heart rates were made with the following results:

H pure—100, 108, 108, 106, 96, 110, 112, 120, 106, 110.
Average 107.6.

H hybrid—100, 130, 148, 120, 116, 100, 130, 120, 122, 124.
Average 121.

M hybrid—74, 110, 82, 96, 110, 116, 56, 100, 96, 90.
Average 93.

M pure—112, 120, 124, 110, 126, 120, 120, 110, 128, 126.
Average 119.6.

In this series the heart rate of the two hybrids were the extremes while the two pure breeds occupied a mean position.

Series E, July 11, 1906. This incomplete series was strikingly in accord with Series 1 as far as it went, but was not followed after 42 hours, at which time drawings of typical specimens of all four strains were made, *H* pure resembling (*h*₄), *H* hybrid resembling (*m*₄) with blastopore almost closed, *M* hybrid resembled (*H*₂), and *M* pure resembled (*h*₂).

Series F, June 24, 1907. This series was followed carefully for four days when it had to be abandoned, on account of absence from the laboratory. At 48 hours this series showed the unusual character of having the *M* pures decidedly in advance of the *M* hybrids. At the end of three days, however, the *M* hybrid had forged distinctly ahead.

Series G, July 8, 1907. No observations recorded except at time of hatching. *H* pures had all hatched at the end of fifteen days while none of the *H* hybrids had hatched. Two days later the majority of the latter had hatched.

Series H, June 23, 1907. *H* pures hatched chiefly on the twelfth and thirteenth days. *H* hybrids hatched as follows: 10 per cent on the thirteenth day, 25 per cent on the fifteenth day, 30 per cent on the sixteenth day. The remaining 35 per cent did not hatch. *M* pures hatched some time after the eighteenth day. About thirty were seen swimming in the dish on the twenty-first day.

Data Derived from the Examination of Preserved Material

Various series were watched and when some doubtful or especially interesting stage was reached a sufficient number of embryos were killed in picro-acetic, and examined afterwards at leisure. Additional and confirmatory data on several points was obtained in this way.

I Rates of Cleavage

1 The eggs of a large *F. majalis* female were divided into two lots of approximately equal size and fertilized simultaneously with the milt of the two species. After three hours development eggs were killed and examined with the following results: *M* pure showed 158 four-cell stages, 2 three-cell stages, 3 two-cell stages, and four uncleaved. *M* hybrid showed: 89 four-cell stages, 2 three-cell stages, 1 two-cell stage, and 114 uncleaved.

2 Another lot of *majalis* eggs treated in the same way. *M* pure 51 four-cell stages, 4 three-cell stages, 3 two-cell stages, and 38 uncleaved. *M* hybrid, 32 four-cell stages, 2 three-cell stages, 1 two-cell stage and 54 uncleaved.

3 Eggs of one *heteroclitus* female used, fertilized with both species of sperm. After three hours there were: *H* pures, 41 four-cell stages, 5 two-cell stages, 54 unsegmented; *H* hybrid, 13 four-cell stages, 3 two-cell stages, 80 unsegmented.

4 Same as 3, but a large lot of mixed eggs from over a dozen females were used. These were divided, after stirring thoroughly into two lots and fertilized with two species of sperm. At the end of three hours they were killed in picro-acetic acid, and examined. Results: *H* pure, 308 four-cell stages, 63 two-cell stages, 563 uncleaved. *H* hybrid, 214 four-cell stages, 18 two-cell stages, 742 uncleaved.

There were probably many unripe and stale eggs in this lot, as no care was taken to select the best males or females.

5 A large number of eggs from a good *H* female were divided into two lots, fertilized with both species of sperm, and after they had reached a stage where there seemed to be about equal numbers of 16 and 32-cell stages the two lots were killed and examined, with the following results: *H* pure, 38 thirty-two-cell stages, 44 sixteen-cell stages, and 29 uncleaved. *H* hybrid, 33 thirty-two-cell stages, 31 sixteen-cell stages, and 42 uncleaved. Of eggs cleaved *H* pure showed 46.3 per cent in 32-cell stage and *H* hybrid 44.6 per cent. The difference was so slight that it was not significant. Four other experiments similarly conducted gave results approximately like those just detailed. In some the percentages lightly favored the pure strain and in others the hybrid strain. The conclusion was reached that there was no measurable difference in time rate of cleavage up to the 32-cell stage, at least.

6 Several experiments were conducted like those just described except that the eggs were allowed to develop until the blastodiscs had begun to spread out over the yolk. They were then killed and examined before any shrinkage had taken place.

a This lot allowed to develop for sixteen hours, and twenty-five eggs, selected at random, were transferred to another vessel, where the blastodisc diameters were measured with ocular micrometer. Results: *H* pure average 20.4 mm.; *H* hybrid 16.6 mm.

b Allowed to develop 14 hours: Average diameter of twenty *H* pures was 16.8 mm., that of *H* hybrids 15.4 mm.

c After 20 hours: Average diameter of twenty *H* pures was 22.6 mm., that of *H* hybrids 20.1 mm.

d After 20 hours: *H* pures 21.3 mm., *H* hybrids 20.8 mm.

7 This series only *F. heteroclitus* strains: After 24 hours the two strains were killed and examined. It was found that the *H* pures were on the average in the condition of (*m*₄), while *H* hybrids were in that of (*M*₃). The difference was very marked.

2 Later Stages

A few detailed camera drawings of whole mounts of later stages were made and a plate of these figures will show better than a

verbal description could, how real a difference exists between pure and hybrid strains at these stages (Figs. 9, 10, 11 and 12).

Set 1: Killed after 56 hours, ten embryos of each strain preserved. Numbers of somites:

H pure: 10, $9\frac{1}{2}$, $10\frac{1}{2}$, 10, 10, $10\frac{1}{2}$, 10, $10\frac{1}{2}$, $9\frac{1}{2}$, 10. Average 10+. (Fig. 9.)

H hybrid: $5\frac{1}{2}$, $7\frac{1}{2}$, 6, none, 6, 4, $6\frac{1}{2}$, $6\frac{1}{2}$, $5\frac{1}{2}$, 6. Average 5.3. (Fig. 10.)

M hybrid: 6, 3, 2, 2, 3, the rest none. (Fig. 11.)

M pure: none in any of the specimens. (Fig. 12.)

The figures show an average condition in each case.

Set 2: Ten of each strain killed after 86 hours. Numbers of somites:

H pure: 21, 21, 21, 21, 20, 21, 21, 21, 21, 20. Average 20.8.

H hybrid: 15, 16, 17, 16, 15, 16, 14, 15, 17, 12. Average 15.3.

M hybrid: 14, 15, 12, 16, 16, 16, 16, 16, 17, 16. Average 15.4.

M pure—13, 13, 13, 13, 13, 13, 12, 13, 13, 12. Average 12.8.

Set 3: Killed after 48 hours, ten embryos of each strain preserved. As there were no somites another index of development was chosen. Four stages of development were arbitrarily chosen and drawn (Figs. 13, 14, 15 and 16). Then the various specimens were referred to the stages by number. When the condition fell between two numbered stages the figure $\frac{1}{2}$ was used to express the intermediate condition. The results were as follows:

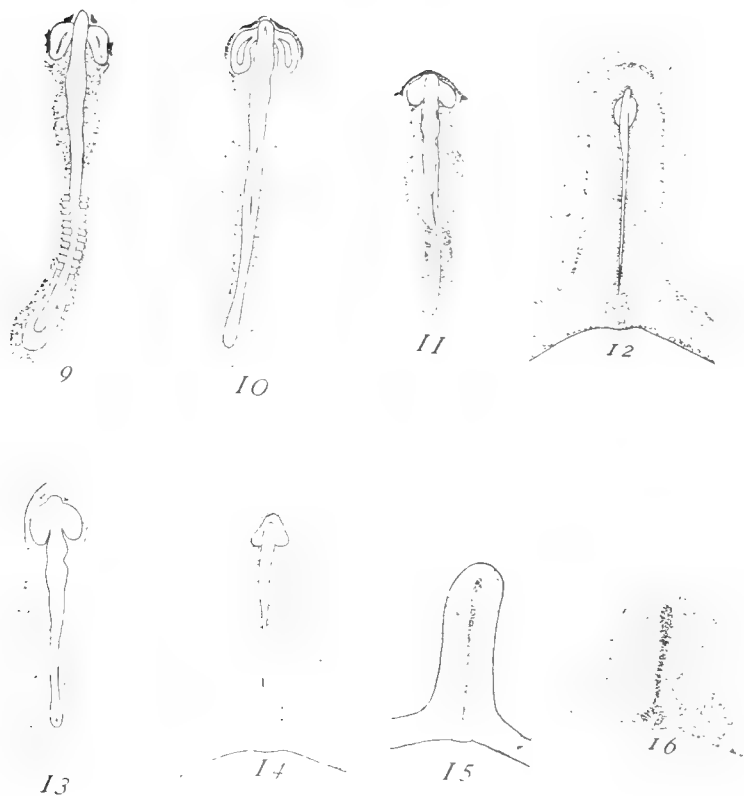
H pure: 4, $3\frac{1}{2}$, $3\frac{1}{2}$, 4, $3\frac{1}{2}$, $4\frac{1}{2}$, $1\frac{1}{2}$, $3\frac{1}{2}$, 4, 4. Average 3.6.

H hybrid: 1, 2, $2\frac{1}{2}$, 4, $2\frac{1}{2}$, 2, 2, $2\frac{1}{2}$, 4, 4. Average 2.65.

M hybrid: 2, $2\frac{1}{2}$, $2\frac{1}{2}$, 2, 1, 2, $1\frac{1}{2}$, 1, 2, 1. Average 1.95.

M pure: 1, 1, $1\frac{1}{2}$, 1, 1, 1, 2, 1, 1, 1. Average 1.15.

Several other sets showed practically the same facts as those just detailed. Later stages do not make favorable whole mounts, on account of their opacity.



Figs. 9 to 12 (inclusive) are camera drawings, showing a magnification of 20 diameters of the four strains at 56 hours.

Embryos were killed and fixed in micro-acetic acid, dissected off the yolk, stained in borax carmine, cleared in bergamot oil, and mounted in balsam.

Fig. 9, average condition in pure bred *F. heteroclitus*.

Fig. 10, average condition in hybrid *F. heteroclitus* ♀ and *F. majalis* ♂.

Fig. 11, average condition in hybrid, *F. heteroclitus* ♂ and *F. majalis* ♀.

Fig. 12, average condition in pure bred *F. majalis*.

Figs. 10 to 16 (inclusive) are camera drawings of embryos prepared as in the series above. The four figures represent the average conditions in four strains of another series at forty-eight hours. The arrangement of figures and magnification is the same as in the series above.

Experimental Data

The facts that the two species show such marked physiological differences and that these differences exhibit themselves when the embryos or adults are subjected to sub-optimal conditions, led to experiments that would afford a comparison of the resistances of the pure and hybrid strains at the same stage of advancement. This type of experiment requires rather large numbers of specimens and a considerable amount of repetition, on account of the high degree of individual variation, especially among the hybrid strains.

I Resistance to Lack of Oxygen

In 1905 considerable time and effort were expended in the endeavor to determine the comparative resistances of the pure and hybrid strains to lack of oxygen. Engelmann gas chambers, boiled sea-water, and a continuous stream of purified hydrogen were used. At that time it was not known that the resistance to lack of oxygen varied with the degree of development, hence a mass of very contradictory data was obtained. The following summer the experiment was repeated several times with embryos of the same age and degree of advancement. The results were quite different from what one would naturally expect. The *H* hybrids proved to be the most resistant, the *H*·pures next, the *M* pures next, and the *M* hybrids the least resistant. The two hybrid strains were the extremes while the two pure strains were the means.

These experiments were so tedious, necessitating watching for nearly twenty-four hours, continuously, or at frequent intervals, that another method of testing the comparative resistance of pure and hybrid strains was tried. This method consisted of putting the embryos in gas chambers divided into four compartments of equal size, filling the chamber with sea-water charged with carbon dioxide, sealing up the chamber and timing the cessation of heart-beat in all specimens. Of course only small numbers could be used, because it was necessary to keep a very close watch over all specimens in order to get even approximately accurate death rates.

Experiment 1, June 26, 1907. The *M* hybrids showed the

most immediate effect of the carbon dioxide, the heart-beat of all five specimens ceasing before the expiration of 5 minutes. The *M* pures lasted, on the average 7 minutes. The *H* pures averaged 18 minutes. The *H* hybrids averaged nearly 30 minutes, all continuing to live after all of the other embryos were dead.

Experiment 2, June 26, 1907. Six embryos of each strain, eight days old. The *M* hybrids all stopped before any of the others. The *M* pures began to show the effect of the carbon dioxide a little later than the *M* hybrids and survived considerably longer. The *H* pures stopped a few minutes later, while four out of six *H* hybrids continued nearly twice as long as the *H* pures.

Experiment 3, July 8, 1907. Ten embryos of each strain used. All 4 days old.

After 5 minutes, 6 *M* hybrids and 5 *M* pures dead.

After 10 minutes, 7 *M* hybrids and 5 *M* pures dead.

After 19 minutes, 8 *M* pures dead, one feeble and one active.

After 21 minutes, 7 *M* hybrids dead, 3 very feeble.

After 28 minutes, all *M* pures and *M* hybrids dead.

After 33 minutes, 4 *H* pures dead, no *H* hybrids dead.

After 38 minutes, 6 *H* pures and 2 *H* hybrids dead.

After 45 minutes, 7 *H* pures dead, 2 feeble, 1 fairly strong; 4 *H* hybrids dead, the rest strong.

After 50 minutes, 9 *H* pures dead, 1 feeble; 4 *H* hybrids dead, 1 feeble, 5 strong.

After 60 minutes, all *H* pures dead; 5 *H* hybrids dead, 1 feeble, 4 strong.

After 75 minutes, 8 *H* hybrids dead, 2 strong.

After 85 minutes, all *H* hybrids dead.

The experiment shows that, in embryos of four days, there was practically no difference between the two majalis strains, but that the *H* hybrids were considerably more resistant than the *H* pures.

Experiment 4, July 9, 1907. An attempt was made to select embryos of the same degree of advancement instead of the same age. The embryos were selected from different series and were

about as advanced as the average *H* pure at 7 days. Ten embryos of each strain were used. Results:

After 5 minutes, 5 *M* hybrids dead.

After 14 minutes, 1 *M* pure dead, 5 *M* hybrids dead.

After 16 minutes, 5 *M* hybrids, 1 *M* pure, and 2 *H* hybrids, dead.

After 22 minutes, 8 *M* hybrids dead, 2 feeble; 2 *M* pures dead; and 2 *H* pures dead.

After 36 minutes, 9 *M* hybrids dead, 3 *M* pures dead, and 2 *H* hybrids dead.

After 38 minutes, all *M* hybrids dead, 4 *M* pures dead and 4 feeble; 5 *H* pures dead.

After 46 minutes, *M* pure 6 dead and 4 feeble; *H* pures 8 dead; *H* hybrids 2 dead.

After 53 minutes, *M* pures and *H* pures all dead; *H* hybrids 6 dead and 2 feeble.

After 60 minutes, 8 *H* hybrids dead, 2 feeble.

After 65 minutes, all *H* hybrids dead.

This experiment shows that in strains of the same degree of advancement the *M* hybrids were the least resistant, the *M* pures next, the *H* pures next, and the *H* hybrids the most resistant.

Experiment 5. Only the two *majalis* strains used; ten embryos of each used; six days old. The *M* hybrids were all dead before any of the *M* pures; three of the *M* pures survived any of the *M* hybrids.

Experiment 6. Only the two *heteroclitus* strains used; ten of each; six days old. Results:

After 5 minutes, 1 *H* hybrid dead.

After 8 minutes, 1 *H* hybrid; and 7 *H* pure dead.

After 16 minutes, 1 *H* hybrid dead; 8 *H* pures dead, 2 feeble.

After 26 minutes, 1 *H* hybrid dead; 9 *H* pures dead and 1 feeble.

After 35 minutes, 2 *H* hybrids dead; 9 *H* pures dead and 1 still feeble.

After 55 minutes, 8 *H* hybrids dead, 2 still vigorous, 1 *H* pure still feebly living.

After 65 minutes all dead.

This experiment shows an interesting fact that there is a well marked individuality among these embryos. One of the *H* hybrids (the more resistant strain) succumbed before any of the *H* pure (the less resistant strain); while one of the *H* pures (the less resistant strain) resisted feebly as long as any of the *H* hybrids. On the whole, however, the *H* hybrids showed themselves to be the more resistant of the two heteroclitus strains.

2 Experiments with KCN

Believing that KCN acts as a reducing agent upon animal tissues and produces the same effects as those produced by lack of oxygen, rather strong solutions of KCN in sea-water were used upon embryos and adults.

Experiment 1. Placed three adult males of each species in a covered vessel filled with one liter of one-tenth molecular KCN solution in sea-water. Results: The three *F. majalis* males died in an average of 16 minutes; the three *F. heteroclitus* males died in an average of 31 minutes. The same experiment was repeated with three females of the two species. Results: *F. majalis* females died in an average of 18 minutes, while the *F. heteroclitus* females died in an average of 30 minutes.

Evidently *F. heteroclitus* is much more resistant to KCN poisoning than *F. majalis*.

Experiment 2. Three six day embryos of each of the four strains put into $\frac{M}{10}$ KCN solution in sea-water:

The *H* pures lived for an average of 93.5 minutes.

The *H* hybrids lived for an average of 82.5 minutes.

The *M* hybrids lived for an average of 70 minutes.

The *M* pures lived for an average of 20 minutes.

Experiment 3. Same conditions as last experiment except that ten embryos of each of the four strains were used. After 2 hours

in the solution they were all transferred to fresh sea-water and allowed to recover in as far as possible. It was found that:

H pures 5 alive and 5 dead, 50 per cent living.

H hybrids 6 alive and 4 dead, 60 per cent living.

M hybrids 8 dead and 2 living, 20 per cent living.

M pures none alive and 10 dead, 0 per cent living.

The experiment shows no marked difference between the two heteroclitus strains, but a more marked difference between the two majalis strains.

Experiment 4. Fifteen of each of the four strains used. Embryos seven days old. Same solution as in Experiment 3.

After $2\frac{1}{4}$ hours' exposure to the poison the embryos were transferred to fresh sea-water and carefully washed. Sufficient time was allowed for all not entirely dead to recover and then the dead were counted:

H pures 12 alive and 3 dead, 80 per cent alive.

H hybrids 6 alive and 9 dead, 40 per cent alive.

M hybrids 1 alive and 14 dead, 6 per cent alive.

M pures 4 alive and 11 dead, 26 per cent alive.

This experiment shows the effect on the heteroclitus strains to be about the same as before, but the conditions for the majalis strains are reversed, the pure strain being the more resistant. The hybrids had begun to show the enfeebling effects of a slower circulation, which finally results in a failure of all specimens to hatch.

Experiment 5. This experiment dealt only with the two heteroclitus strains. Embryos nineteen days old. Same solutions as before. The *H* hybrids showed the first effects of the poison, four dying before any of the *H* pures. Three *H* pures survived all of the *H* hybrids.

The experiments with KCN show at least one thing, viz: that the introduction of *F. majalis* sperm into the egg of *F. heteroclitus* produces a hybrid with a lessened resistance to this particular poison.

IV SUMMARY OF DATA

1 The volume of *F. majalis* eggs is on the average more than twice that of *F. heteroclitus*. The rate of development is rather closely proportional to the mass of the eggs, the smaller egg developing nearly twice as fast as the larger egg.

2 The yolk of *F. majalis* eggs is much more yellow and opaque than that of *F. heteroclitus*. This optical difference undoubtedly indicates a much more deep seated difference in chemical composition.

3 A far larger percentage of eggs are fertile to sperm of their own species than to that of foreign species.

4 The rate of early cleavage is not measurably altered by the introduction into the egg of sperm belonging to a more slowly or more rapidly developing species.

5 The earliest measurable effect of foreign sperm in hastening or retarding development is seen in the egg of *F. heteroclitus*. At periods ranging from fourteen to twenty hours the blastodisc of *H* pure shows a measurably greater diameter than that of *H* hybrid. In the eggs of *F. majalis* this difference is not seen for an average of about six hours later, but it is just as marked when it appears.

6 In general the development of *F. heteroclitus* eggs is retarded by the introduction of *F. majalis* sperm, while that of *F. majalis* eggs is accelerated by the introduction of *F. heteroclitus* sperm. This acceleration or retardation is not permanent for either of the hybrid strains. The more fortunate of the *H* hybrids, although retarded for the first eight or ten days, at and subsequent to hatching are somewhat larger, have a more rapid and more efficient circulation, are more active in their movements, show a greater resistance to lack of oxygen and the presence of carbon dioxide, and live much longer in captivity, than do any of the *H* pures. The *M* hybrids, on the other hand, develop more rapidly than do the *M* pures for a period of from seven to ten days, but after that they gradually cease to grow, attain a size only about half that of the *M* pures at hatching (but about equal to that of the *H* pures at the same period) and never succeed in hatching because of their

apparent inability to consume the large mass of superfluous yolk.

7 The heart-beat of the *H* pures appears about ten hours earlier than that of the *H* hybrid and this gives the former a decided advantage over the latter in rate of subsequent development. When, however, the *H* hybrids attain a heart-beat and a circulation it is a more rapid and efficient one than that of the *H* pures (the more rapid heart-beat being an endowment from the paternal species), and they rapidly overhaul the *H* pures and, for a time, seem to be almost on an even footing with the latter, a small percentage of them certainly surpassing any of the *H* pures. The heart-beat and circulation of *M* hybrids appears nearly a day earlier than that of the *M* pures, and for a time the former show a decided advantage over the latter. But when the *M* pures attain a heart-beat and circulation it is a more rapid and efficient one and the *M* pures overtake and pass the *M* hybrids. The latter remain behind permanently and, with their slower heart-beat and less efficient circulation they never succeed in consuming more than half of the yolk with which the egg is endowed.

8 The phenomenon of pigmentation runs parallel with that of circulation and is probably dependent to some extent thereon. The *H* pure embryos become heavily pigmented in about three days, while the *M* pures become pigmented very late in development, and then comparatively lightly. Naturally the *H* pures show the first signs of pigmentation, dark brown chromatophores appearing on yolk and body soon after the establishment of a circulation. About a day later the *H* hybrids begin to show signs of pigmentation, but at first far less abundantly than the *H* pures. In later stages, however, the most successful of the *H* hybrids surpass in depth of pigmentation the most heavily pigmented of the *H* pures. This may be explained by the fact that these hybrids combine the *F. heteroclitus* character of densely packed chromatophores with the *F. majalis* character of a darker colored pigment. The *M* hybrids show a much earlier and much heavier pigmentation than do the *M* pures. The difference at about five days is very evident to the naked eye. The color pattern on the head and trunk, characteristic of *F. heteroclitus* embryos, is

found on all embryos of the *M* hybrid at the period of their maximum development. The same pattern is found on all successful *H* hybrids embryos in advanced stages.

9 The chromatophores of *F. majalis* are small-bodied and finely branched, while those of *F. heteroclitus* are proportionally larger bodied and much less finely branched. The *F. heteroclitus* type of chromatophore is found in three out of the four strains, viz: *H* pure, *H* hybrid, and *M* hybrid.

10 The size of *F. majalis* on hatching is about twice that of *F. heteroclitus*. That of *H* hybrids is, on hatching, no larger, on the average, than that of *H* pures. The maximum size reached by the *M* hybrids is about equal to that of the *H* pures on hatching.

11 The examination of preserved material furnished more easily measurable data for demonstrating the differences at various stages of development between pure and hybrid strains. The numbers of mesoblastic somites at various stages offers an accurate and convenient measure of stages of development. In every case examined the data derived from preserved material served to confirm observations made upon living material.

12 The hybrid strains, especially the *H* hybrids, showed a remarkable degree of variability, far wider in range than was seen in the pure strains.

13 In the experiments involving the lack of oxygen and the presence of carbon dioxide, the two hybrid strains, instead of occupying positions between the two pure strains, fell to the extremes. The *H* hybrids were the most resistant, the *H* pures next, the *M* pures next, and the *M* hybrids the least resistant.

14 KCN solutions in sea-water were found to act in quite a different way from carbon dioxide or lack of oxygen. Here the hybrid strains seemed to occupy mean positions, although with no great degree of regularity. The data derived from the use of KCN was not very satisfactory.

V DISCUSSION

The Relative Influence of Maternal and Paternal Elements in Determining the Characters Seen in Early Development

Driesch,⁴ as the result of his hybrid experiments with various species of echinoids, came to the conclusion that the early development of hybrids is practically determined by the character of the egg protoplasm; that the rate of cleavage, the number of primary mesenchyme cells, the form of the larval body, and the color content of the hybrid tissues, are exclusively maternal characters, and that no male influence is felt until a comparatively late period, when the larval skeleton is forming. These rather sweeping conclusions were subsequently modified to a slight extent in response to a criticism by Boveri.⁵ Driesch⁶ admitted that at least one of the characters claimed by him to be pure maternal, showed the male influence. This was the character of intensity of coloration of the hybrid larvæ. Further than that he was unwilling to go.

Boveri showed that the number of mesenchyme cells is noticeably influenced by the male parent. In fact, it appears from Boveri's work that the form of larval body and almost every character of the hybrids show the paternal influence. It was admitted, however, that the chief controlling factor in early development is the egg protoplasm.

Driesch and Boveri have never reached an entire agreement on this matter. They stand for two opposite doctrines of heredity. Boveri is a stanch champion of the idea that the nucleus is the sole bearer of hereditary material and hence has a tendency to overemphasize the paternal influence, since he believes the sperm cell to consist almost entirely of nucleus. Driesch, on the other hand, believes firmly that the cytoplasm is of equal value in inheritance, and somewhat oversteps the mark in claiming that the egg protoplasm is the sole factor in determining the character of the phenomena of early development.

The data advanced in the present paper lead to a middle course. For merely mechanical reasons the egg protoplasm must necessarily

⁴ Driesch, H. Arch. f. Entw.-Mech., 7, 1898.

⁵ Boveri, Th. Arch. f. Entw.-Mech., 16, 1903.

⁶ Driesch, H. Arch. f. Entw.-Mech., 16, 1903.

determine some of the characters of the hybrids, such as the maximum size on hatching, the rate of early cleavage, and the degree of transparency of the embryo and of the yolk sac. It is scarcely to be expected that a mass of protoplasm and yolk, such as the fish egg, should show an immediate measurable alteration on the entrance of a body comparatively so minute as the fish sperm cell. It naturally takes time for the sperm cell to reorganize the comparatively enormous mass of egg protoplasm to such an extent that the rate of the developmental process is measurably altered. As a matter of fact it should cause some surprise that the sperm cell is able to do this work in so short a time as it does. The data cited above show that there was a distinctly measurable retardation of the developmental process in the case of *H* hybrids after a period as brief as fourteen hours, a time very short as compared with the total period of embryonic development. It is practically certain that this influence of the sperm cell was operative at a much earlier period, even though it was not measurable by the crude methods applied.

The only pure maternal characters that have been noted are some of the characters that exist before the influence of the sperm could reasonably be expected to make itself felt, and a few mechanically determined characters such as have been mentioned. In all other characters the sperm influence was evident in some form or other.

These results and conclusions are out of accord with the results of Loeb⁷ and Godlewski,⁸ who found that the larvæ, produced by fertilizing sea-urchin eggs with the sperm of holothurians and crinoids, showed only sea-urchin (maternal) characters.

It seems necessary to take exception to a statement made by Conklin⁹ in a paper that appeared since this work went to press, "that the early development of animals is of purely maternal type, and that it is only in stages later than the gastrula, and consequently after the broad outlines of development and the general

⁷ Loeb, J. University of California Publications, vol. i, 1904.

⁸ Godlewski, E. Arch. f. Entw.-Mech., 20, 1906.

⁹ Conklin, E. G. Science, vol. xxvii, no. 681, January, 1908.

type of differentiation have been established, that the influence of the spermatozoön begins to make itself felt." In the present work I believe that the evidence shows that the influence of the spermatozoön is probably immediate and becomes distinctly measurable long before gastrulation.

Exclusive versus Blended Inheritance

Are there any examples of exclusive inheritance in the present work? A pure maternal character, such as the rate of early cleavage, might be termed a dominant character, but if we admit it to be such, we have not far to go in search of a physical explanation of this dominance.

Nearly all of the characters observed may be classed as examples of blended inheritance of one sort or another. The only characters that cannot be thus classified, but seem to be akin to exclusive inheritance are:

1 The size of the hybrids of both strains on hatching. The size of the *M* hybrids at the period of maximum development, which is the equivalent of the stage of hatching in the other strains, is on the average that of the smaller species, *F. heteroclitus*, although the hybrids have developed within eggs of *F. majalis*, the larger species. The *H* hybrids are on the average only equal in size to the *F. heteroclitus* parent at the same period. The character of size on hatching might be called a dominant *F. heteroclitus* character, for all strains containing *F. heteroclitus* are of approximately equal size on hatching (see Plate V).

2 The color pattern on head and body of both reciprocal crosses is, at a period shortly before and after hatching (in *M* hybrids at the period of maximum development) practically identical with that of the *H* pure. *F. heteroclitus* might then be called dominant with regard to the color pattern at this developmental period (see Plate V).

Another kind of inheritance occurs, which is neither exclusive nor blended, but seems to be a sort of an exaggeration of exclusive inheritance, if we may be pardoned the expression. Cases occur in which the hybrids carry certain characters to extremes, showing them to a more marked degree than either parent species. We

might call such a condition *hyper-dominance*. Some examples of this phenomenon follow:

1 When all four strains of a series were subjected to the experimental conditions of lack of oxygen or the presence of carbon dioxide, it was found that the *H* hybrids were the most resistant, the *H* pures next, the *M* pures next, and the *M* hybrids the least resistant. The *H* hybrids then carry resistance to an extreme while the *M* hybrids carry lack of resistance to an extreme.

2 A somewhat similar condition was seen in the degree of depth of pigmentation of many of the *H* hybrids just before and just after hatching. A considerable percentage of the latter become more darkly pigmented than any of the *H* pures (the darker parent species).

3 After hatching it was found that the more successful type of *H* hybrids grew faster and lived far longer, under the conditions applied, than any of the *H* pures (the parent species that lives the longer under these conditions).

That there were all degrees of this dominance or hyperdominance cannot be denied, but the same can be said of practically all cases of dominance described in the literature. There really appears to be no valid reason why these cases just cited should not be dealt with as examples of the phenomenon of dominance or exclusive inheritance. I think, however, that I can demonstrate that these cases of dominance are the secondary physiological results of the interaction of two sorts of blended inheritance, viz: in the time of establishment of the heart rhythm and in the rate of heart-beat, both of which affect the general circulation.

The heart rhythm of *H* pure, in accordance with the more rapid development of this species, is established about 24 hours earlier than in *M* pure. Both reciprocal crosses show an intermediate condition, a blending more or less complete, with regard to this character of time of establishment of heart rhythm.

In the case of rate of heart-beat the following are the facts: the rate of heart-beat in *F. heteroclitus*, after it has been well established, is much slower than that of *F. majalis*, that of the former being on the average about 135 beats per minute and that of the latter about

105. The individuals of both hybrid strains show all degrees of blending of these two rates, each strain showing an average not far removed from the ideal mean, 125 beats per minute.

The effects of these two cases of blending are far-reaching, and are quite different in the two hybrid strains.

The *H* hybrid strain shows the *F. majalis* influence in acquiring a heart rhythm about ten or twelve hours later than the *H* pures. The process of development in an embryo is greatly accelerated by the establishment of a circulation, and as a consequence the *H* pure strain gains markedly upon the *H* hybrids at this time (compare *H*5 and *h*5, Plate III). When, however, the heart rhythm of the *H* hybrids is established, it is distinctly more rapid on the average than that of the *H* pures and this greater rapidity, other things being equal, initiates more rapid development. Consequently the speed of development, previously retarded by the belated establishment of the heart rhythm, is now accelerated by the introduction of a more rapid heart rate, and, as a result, all of the healthy *H* hybrids gain markedly upon the pure bred embryos and present, after a period of eight or ten hours, the relative average conditions seen in *H*6 and *h*6 (Plate III). The hybrid specimens that inherit the least retardation in the establishment of a heart rhythm and the greatest acceleration in the rate of heart-beat, actually overtake the best of the *H* pures before hatching, a few hatching as early as any of the *H* pures. After hatching these same fortunate hybrid specimens outgrow and outlive the best of the *H* pures, and these are the specimens that show the most marked condition of hyperdominance with regard to rate of development and resistance to adverse conditions. The specimens which, on the other hand, have inherited the maximum retardation in the establishment of a heart rhythm and the minimum acceleration in the rate of heart-beat are too severely handicapped to succeed in the race, and they lag far behind, and become weaklings or monstrosities, carrying the character of ill-health to an extreme not met with in either of the pure breeds. This condition might also be considered as a case of hyperdominance.

The case is entirely different for the *M* hybrids. Here also we

have a blending both in the time of establishment and the rate of the heart rhythm. The *M* hybrids acquire a circulation at least twelve hours earlier than the *M* pures, but it is a slower and less efficient circulation. For a time the *M* hybrid gains markedly upon the *M* pure, but the slower heart rhythm seems to make it impossible for the circulation to incorporate more than about half of the yolk and while the *M* pures go ahead and develop into large, normal young fish the *M* hybrids reach a stage as advanced as that at which the embryos of other strains hatch, but are left stranded on a mass of yolk that the metabolic processes are unable to cope with. The fact that the size of these abnormal embryos is about equal to that of the *H* pures on hatching may be simply a coincidence and not a case of dominance at all.

In the case of the *H* hybrids the size on hatching is, as was previously stated, simply a physical necessity since the egg membrane can contain an embryo of only a definite maximum size.

The darker coloration of many *H* hybrids may also be explained as a result of the blending of two characters. These individuals combine the heavy-bodied type of chromatophore seen in *H* pure, the closer aggregation of the latter (also a heteroclitus character), with the darker pigment of the *F. majalis* parent. This combination gives the impression of a decidedly darker coloration. Of course there are all degrees of blending with regard to the shape and closeness of aggregation of the chromatophores, and in the depth of color in the pigment, but those individuals that possess the highest degree of similarity to the parent species in all three respects give the impression of being more darkly colored than either of the parent species.

The case of hyperdominance involved in the comparative resistances of hybrid and pure strains to lack of oxygen and the presence of carbon dioxide, may be explained as the physiological result of the blending seen in the heart rhythms. The *H* hybrids, being endowed with a more rapid and hence more efficient circulation than the *H* pures, are enabled to withstand and throw off the effects of the accumulating carbon dioxide in the system more successfully than the *H* pures. On the other hand the *M* hybrids, handicapped by a slower and less efficient circulation than the

M pures, and having an equal amount of material to assimilate, succumb more readily to accumulations of carbon dioxide than do the *M* pures.

No physiological explanation is offered for the dominance of *F. heteroclitus* in the matter of color pattern at the time of hatching. This seems to be a case of dominance much like many that have been described in other forms in connection with definitive characters.

Dominance and Survival

Is dominance the equivalent of survival? This is a question difficult to answer with assurance. It appears, however, that the *H* hybrid individuals showing the highest degree of dominance in characters that are of vital significance, such as time of establishment of heart rhythm and rate of heart-beat, produce at one end of the series individuals that survive and outlive the best of the pure bred individuals of either species; and at the other extreme of the series, individuals that fail to survive, perishing during the developmental process. Thus dominance of certain good combinations of characters means survival, and recessiveness of these same combinations means early death.

In a sense then the dominants, those with the most nearly perfect resemblance to one parent in a certain set of vital characters are the only ones that survive; the recessives, those with the least perfect resemblance, perish early in the developmental process. All the survivors show dominance with regard to certain combinations of characters, and, if we were dealing only with end results we would probably be deceived into considering that we had a typical case of exclusive inheritance. But we have the advantage of having seen the elimination of the recessives.

We may conclude, then, that the dominance of good combinations of characters is the equivalent of survival, while the recessiveness of these same combinations of characters is the equivalent of failure to survive. In this sense our question "Is dominance the equivalent of survival?" is answered in the affirmative.

The dominance of superficial, non-vital characters, such as color pattern, intensity of pigmentation, etc., may be definitely

correlated with the dominance of certain good combinations of vital characters. For example it appears that intensity of pigmentation is, in the *H* hybrid strain, associated with unusual rapidity of heart-beat.

Might we suggest the possibility that cases of dominance in other experimental fields might be explained physiologically in some such way as has been suggested in the foregoing discussion?

High Degree of Variability in Hybrid Strains the Result of Varying Degrees of Compatibility in the Germ Cells of the Two Species

That hybrids are more variable than pure breeds is a fact long established and is only emphasized by the data brought out in this paper. The range of variability is, however, far wider than might be supposed were only the successful types of hybrids considered. The following series of types showing the various degrees of success in development of *H* hybrids may prove instructive:

- 1 Many eggs never cleave although fertilized by foreign sperm.
- 2 A few cleave but break down in the two, four, eight, or sixteen-cell stages, through some disharmony in the germinal materials involved. This disharmony is usually noticed in one cell, from whence it spreads through all the cells of the blastodisc.
- 3 Many embryos die and disintegrate in advanced blastodisc stages.
- 4 A small percentage of embryos are weaklings, due to some incompleteness in organization. Many of them fail to establish a circulation and die at an early age. Others reach a stage similar to that of an *H* pure at 48 hours and then die.
- 5 A large percentage of embryos develop into incomplete and monstrous forms, such as those shown in Figs. 3 to 8. These show all grades of incompleteness and discord of parts.
- 6 A few embryos develop to full size but never hatch unless released artificially from the egg membrane. Even then they are sluggish and soon die.
- 7 A large percentage of embryos are rather pale in color, hatch rather late, and live only a few days, but do not grow.

8 About 10 per cent of the embryos are larger and more active than the pure bred individuals, hatch early, some few as early as the earliest of the pure breeds, are darker in color than the latter, have a more efficient circulation, outgrow and outlive the best of the pure strain.

These last individuals are those in which the blending of characters has produced the most harmonious result.

We see then that there are all degrees of compatibility of germinal material from an entire failure to unite in fertilization to the production of a type of hybrid that is better fitted to cope with its environment than either of the pure strains.

The Rhythmic Flux of Characters

Many of the facts brought out by the data presented serve to emphasize the idea of heredity as a process. Instead of a fixity of relationship between pure strains and hybrids, there is a constant flux. At one time we see the paternal influence in the ascendancy, only to be overshadowed later on in development by the maternal influence. If we were to make the statement that in *M* hybrids the presence of heteroclitus sperm accelerates the process of development, we would be overlooking the fact that this influence is at best transitory, that the acceleration is evident only after about twenty-four hours of development, and that, after a period of from seven to ten days the *M* pure strain develops more rapidly than the *M* hybrid. The latter gradually loses its developmental momentum and comes to a complete stop, due probably to its inability to handle a large mass of yolk that remains unassimilated.

In the case of the *H* hybrids, too, we would be wrong if we stated unqualifiedly that the introduction of *F. majalis* sperm retards development. In the first place no measurable retardation is evident until a lapse of about sixteen hours, on the average. In the second place the more successful of the hybrids overtake the average *H* pures after six or seven days, and although the majority of the hybrids subsequently lose ground, a few maintain their advantage and after hatching outgrow the best of the pure strain.

There is no fixity of characters here, but everything is flux and

change. A character then may be conceived of as going through a series of conditions before reaching the definitive state. It may have started out as a dominant, become recessive for a time, dominant again, and so on for a varying number of alternating phases; and who knows whether the characters that we sometimes call definitive are really the end stages in the process? Possibly the further development of the individual as it reaches maturity or senescence may show a condition of dominance less dominant or even recessive, while some recessive characters may come to light that before were unsuspected. If development is continuous as long as life exists, and we have no reason to doubt that such is the case, we should be somewhat cautious about our statements with regard to the permanent dominance of this or that character. If we limit our statements to some particular period, such as early maturity or a larval condition, we would avoid the danger of overstatement.

The Importance of External Factors in Heredity

Experience has taught us that only when we make every effort to equalize the external conditions of a breeding experiment can we expect to get anything approaching uniformity of development. Slight differences in the physiological condition of the parents, varying degrees of freshness of eggs or sperm, differences in temperature and of water content, tend to produce differences in the developmental process that cannot be attributed to the introduction of foreign sperm. We are then driven to the conclusion that uniformity of external conditions is as important a factor in determining a similarity of offspring to parents as is uniformity of germinal substance.

Heredity is defined simply as a similarity of offspring to parents. The question arises as to what constitutes this similarity. If the germ cells from which the offspring develops are similar to those from which the parent developed, and at the same time the external conditions of development are similar, there results invariably a similar developmental process, during which the offspring resembles the parent stage for stage. This similarity of developmental process, it seems to me, is the essence of heredity. The condition-

ing factors of a similarity of process are, first a similarity of germinal substance, and second a similarity of external conditions. Whether these determining factors are of equal or unequal potency is a question that scarcely requires an answer. Neither is operative without the other, and in this sense they are of equal potency. Any change in either will produce a change in the process of heredity, and to that extent a dissimilarity between parent and offspring, which means a failure to accomplish an ideal heredity. Ideal heredity can never be realized because of the inherent variability of things. No two organisms ever start out from identical germ cells, nor do they develop under identical external conditions. Therefore a striking degree of similarity between parent and offspring is all that is ever realized.

If then we admit that the essence of heredity is the similarity of developmental process, we must come to the conclusion that the study of development and of heredity are identical, except in that the latter is a comparative study, the object of which is to determine to what extent the developmental phases of offspring resemble those of parent, or as in the present work to determine what relative influence is to be attributed to either parent.

Heredity has long been studied as a static condition instead of a dynamic process, and it is the hope of the writer that the work here presented (itself somewhat crude and incomplete) may open the way to more and better researches in this most interesting field.

EXPLANATION OF PLATES

PLATE I

Adults of both species of *Fundulus* used in the experiments. Drawings were made from life by Miss Ella Weeks of the State Agricultural College of Kansas, and were first published by the writer in a paper, entitled "Spawning Behavior and Sexual Dimorphism in *Fundulus heteroclitus* and *Allier Fish*," which appeared in the *Biological Bulletin*, vol. xii, no. 5, and was kindly loaned by the editor for the present use.

The figures are life sized representations of average adults as they appear during the spawning season. The pronounced sexual dimorphism is well shown. The larger size of *F. majalis* should also be noted.

Fig. 1. Adult female *F. heteroclitus*.

Fig. 2. Adult male *F. heteroclitus*.

Fig. 3. Adult female *F. majalis*.

Fig. 4. Adult male *F. majalis*.

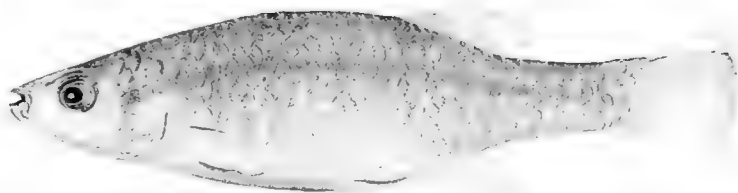


FIG. 1

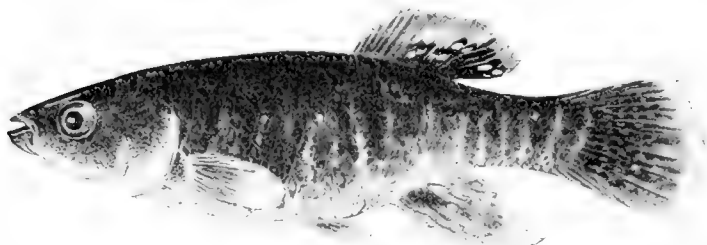


FIG. 2

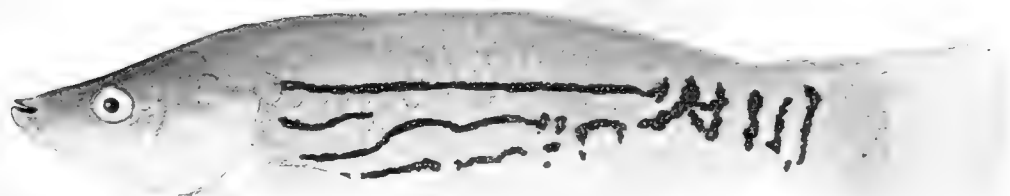


FIG. 3

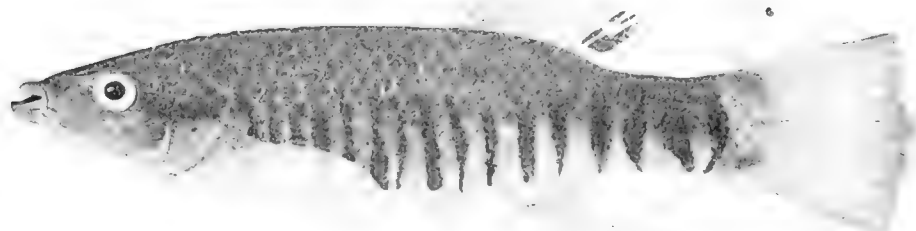


FIG. 4

PLATE II

Pictorial table, illustrating three stages of the Type Series (Series I).

The top row, *H*, in this plate and in the two succeeding plates, depicts stages in the development of pure *F. heterochitus*.

The second row, *h*, represents simultaneous conditions in the hybrid strain *F. majalis* ♂ and *F. heterochitus* ♀.

The third row, *m*, simultaneous conditions in the reciprocal cross *F. heterochitus* ♂ and *F. majalis* ♀.

The bottom row, *M*, simultaneous conditions in pure *F. majalis*.

All figures are camera drawings except that the egg membrane has been drawn in with the compass. All figures show a magnification of 12 diameters.

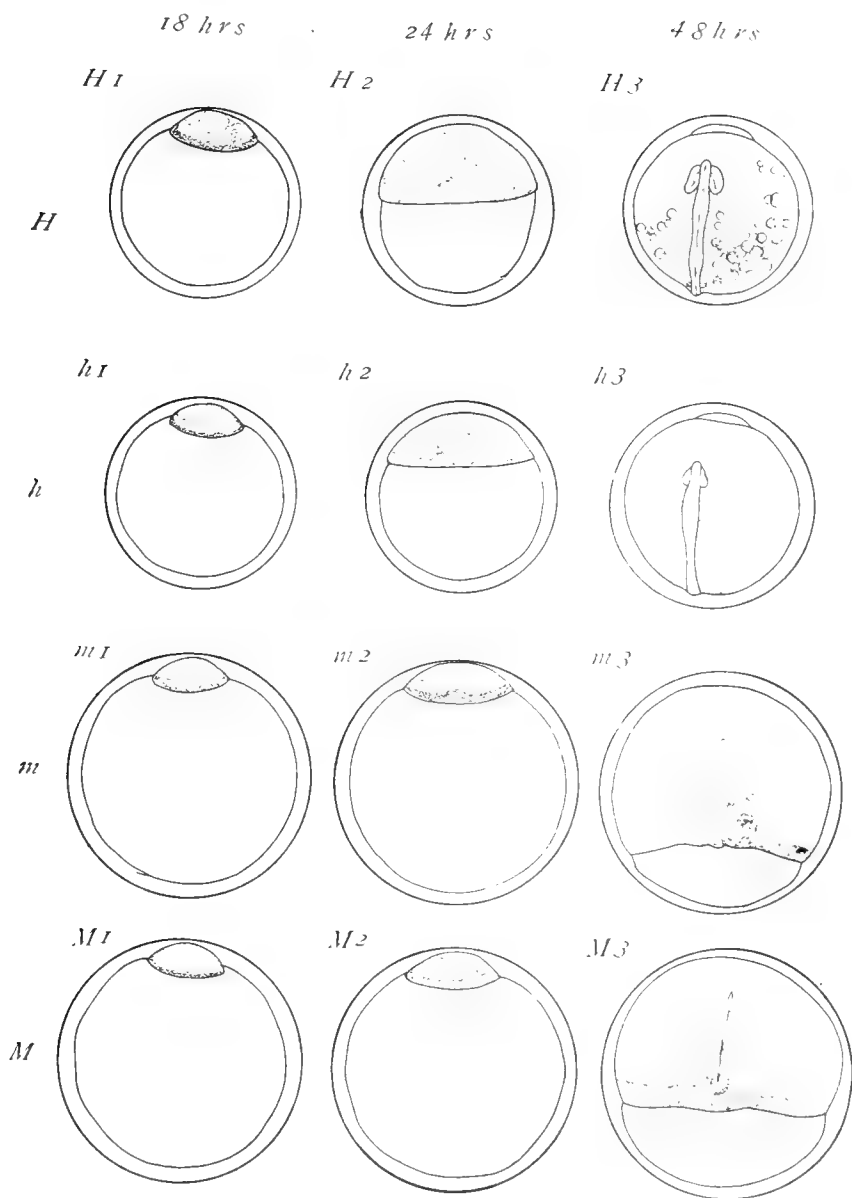


PLATE III

A continuation of the series begun in Plate II. The significance of arrangement and labeling of figures is explained in the legend of Plate II.

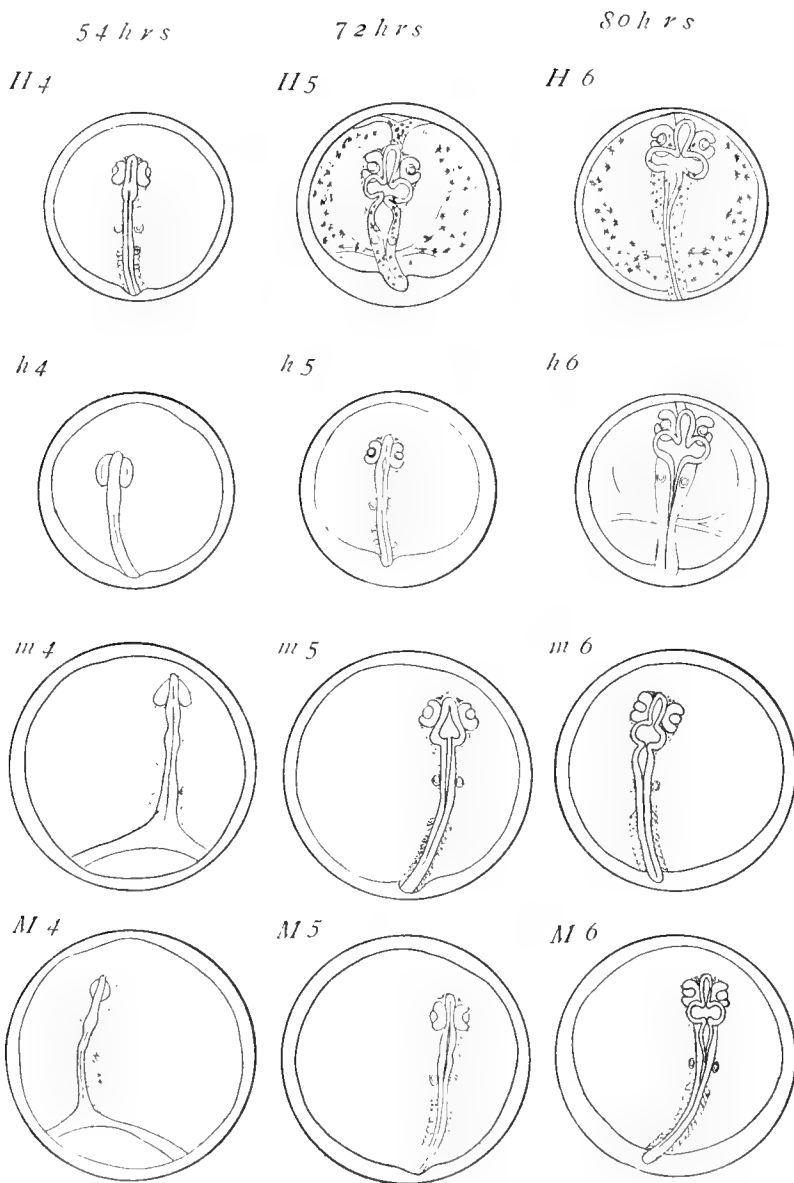


PLATE IV

A continuation of the series treated in Plates II and III. Labeling and arrangement of figures explained in the legend of Plate II.

96 hrs

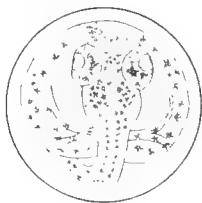
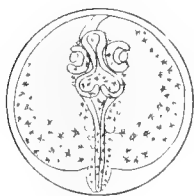
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H7

H8

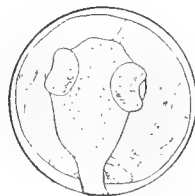
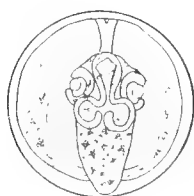
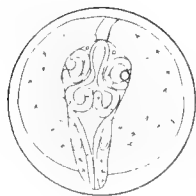
H9



h7

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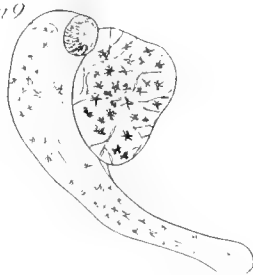
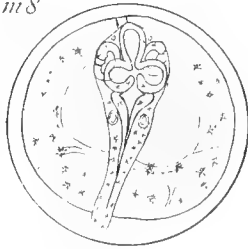
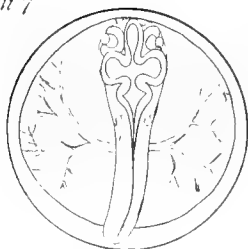
h9



m7

m8

m9



M7

M8

M9

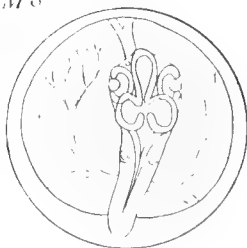
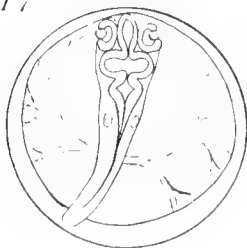


PLATE V

Camera drawings of typical specimens of the four strains of Series I on hatching or at a period equally advanced.

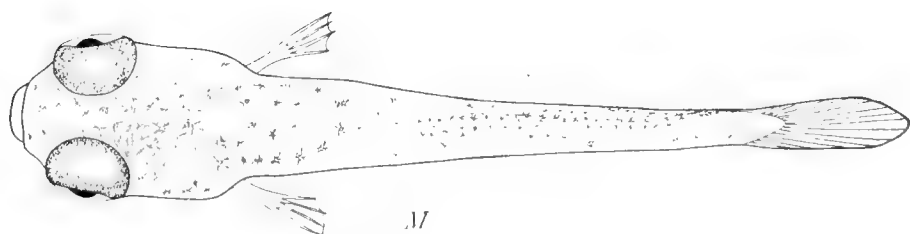
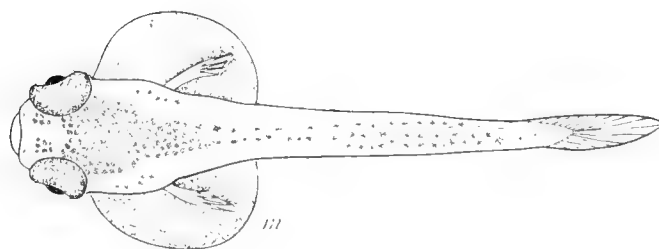
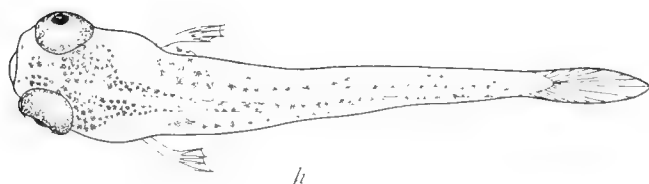
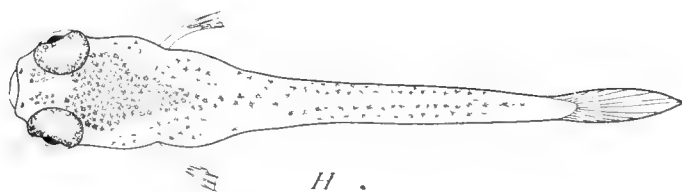
H represents a typical specimen of pure bred *F. heterochitus* on hatching.

h represents an average specimen of the hybrid strain, *F. majalis* ♂ and *F. heterochitus* ♀.

m represents a well developed specimen of the hybrid strain *F. heterochitus* ♂ and *F. majalis* ♀ after 27 days development, after nearly all of the pure *F. majalis* strain had hatched. The size and general appearance of this type of hybrid is almost identical with those of the paternal species, *F. heterochitus*, although the embryo developed from a *F. majalis* egg. The large mass of surplus yolk, which the embryo is unable to assimilate is seen beneath the head and trunk.

M represents a typical specimen of pure bred *F. majalis* on hatching.

All figures drawn to the same scale, showing a magnification of 12 diameters.





FURTHER RESULTS OF TRANSPLANTATION OF OVARIES IN CHICKENS¹

BY

C. C. GUTHRIE, M.D., PH.D.

WITH THREE FIGURES

INTRODUCTION

Numerous attempts have been made to transplant ovaries in animals with the view of studying their function after the operation. In general, such attempts have been unsuccessful.

In 1903 Prof. E. P. Lyon, then at the University of Chicago, invited me to join him in studying ovarian transplantations in bitches. Feeling confident that the operations would be unsuccessful with the surgical facilities at our command, and knowing of the high resistance of fowls to infection and to surgical shock, I suggested the use of chickens.²

EXPERIMENTS

We operated upon two adult hens but the results were unsatisfactory. It was in the laying season (February, 1904) and we attributed the operative results to the unfavorable condition of the ovaries. We then determined on using young chickens but before we could carry out this intention Professor Lyon assumed the direction of another laboratory and was unable to continue in the work.

During the summer of 1904 (August 18) I exchanged the ovaries between two black and two white leghorn pullets, weigh-

¹ A preliminary report was made before the American Physiological Society in Washington City, May 7, 1907. (See Proceedings of the Society, American Journal of Physiology, July, 1907, vol. xix, pp. xvi-xvii). Results of this work was also reported at the Seventh International Congress of Physiologists, Heidelberg, August 13-16, 1907. (See Archives Internationales de Physiologie, 1907, vol. v, p. 108.)

² Since this paper was written I have learned that Dr. E. P. Lyon, even before my acquaintance with him, planned a similar series of experiments on pigeons and even made some introductory experiments on them. His reason for selecting the pigeon was that it has but a *single* functional ovary.

ing about 650 gms. each. One black and one white pullet were saved for controls. All did well for some time after the operations but during the winter, before the laying season began, their condition became extremely poor owing largely to being kept in inappropriate quarters. They lived until the following September but no eggs were laid, even by the controls. During my absence after this date, all but one of the hens were lost, including the controls. The remaining operated hen was killed about October 15, and preserved.

In size and external appearance the hen that was killed seemed normal. Macroscopically, the ovary appeared normal in size, location and in its relations to the surrounding structures. It was many times larger than when transplanted. At the time of the operation the Graafian follicles did not exceed 1 mm. in diameter while postmortem they ranged from 5 to 10 mm. No corpora lutea were observed. Histologically, the ovary appeared normal. The hen was found to be affected with tuberculosis and the ovary was involved, as evidenced by the discovery of a few of the bacilli in the stroma.

On August 25, 1906, another series of pullets of the same strains³ were similarly operated upon, controls being saved as before. They weighed about 750 gms. each, the white ones being slightly the heavier. All did well after the operation. About August 31, they were sent to Columbia, Mo., where they were kept in a small poultry yard until January 21, 1907, when they were shipped to this laboratory.⁴ They have since been kept in individual pens. The roosters have been kept in small slightly closed cages except for the time they have been placed in the pens to tread the hens. Great care has been exercised to keep the records correct in all respects.

SUMMARY OF RESULTS

No marked differences in egg production were found between the control and operated hens, nor in the fertility of the eggs.

³ All of the chickens of the single comb black and white leghorns used to date, including the rooster, have been purchased of E. G. Wyckoff, Ithaca, N. Y.

⁴ Excepting *W*₃, as per addenda to table.

The eggs⁵ and chicks averaged less in weight from the operated hens than from the controls.

The operated hens at the beginning of the laying season were somewhat lighter than the controls.⁶ In other respects no differences were observed either in the hens, eggs or chicks.

The eggs became fertile in two to four days after mating. On cessation of mating the eggs became infertile in eleven to nineteen days, the majority becoming so on the fifteenth day.⁷

Control hens (B_1 and W_1), mated to the rooster of the same breed gave uniformly black fetuses and chicks in the case of the black hen, and white fetuses and chicks in the case of the white hen.

The normal black chicks had grayish-yellow breasts and throats and frequently the under surface of the tips of the wings were light colored as well, but the plumage on the entire dorsal surface was *always* solid black. The light colored areas on the

⁵ Table showing weights of eggs

Black hen no. 1 (B_1) gms.	White hen no. 1 (W_1) gms.	Black hen no. 2 (B_2) gms.	White hen no. 2 (W_2) gms.	Black hen no. 3 (B_3) gms.	White hen no. 3 (W_3) gms.
58.0	60.5	43.5	49.0	43.5	45.0
62.5	57.5	36.0	49.0	45.0	49.0
63.0	58.5	38.5	50.0	51.0	46.0
55.5	60.0	41.5	46.0	48.5	49.0
59.5	61.0	42.5	49.0	44.5	51.5
62.5	57.0	43.5	52.0	47.5	51.5
Aver. 60.1	59.1	40.9	49.2	46.6	48.6

Note—The eggs in this table were laid early in March.

⁶ February 14, 1907, the weights of the chickens were as follows: grams.

Black hen no. 1 (B_1)	1500
Black hen no. 2 (B_2)	1250
Black hen no. 3 (B_3)	1250
Black rooster (BR)	1650
White hen no. 1 (W_1)	1480
White hen no. 2 (W_2)	1450
White hen no. 3 (W_3)	Ill. Not less than 1250
White rooster (WR)	1990

⁷ This agrees with results on this point published by the Ohio University and Experiment Station.

ventral surface were uniformly black after the first moult. Occasionally a normal black may retain one or several white feathers in the tip of the wing permanently, but this is of rare occurrence and such white feathers have not been observed in any other situation.

The normal white chicks were pure white to light buff when hatched but after the first moult they were always pure white.

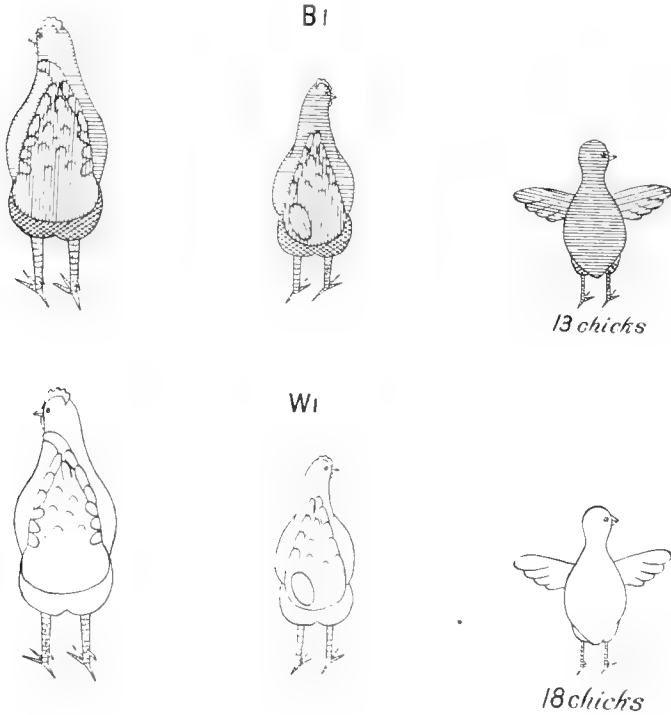


Fig. 1 Controls

The black hen (B_2) carrying an ovary from a white hen (W_2) mated to the white rooster, gave about equal numbers of white and spotted fetuses and chicks. (In all cases of *very small white fetuses*, spots may have been overlooked.)

The white hen (W_2) carrying an ovary from a black hen (B_2) mated to the white rooster, gave white, black and spotted fetuses and chicks. The spotted ones outnumbered the others combined.

The black hen (B_3) carrying an ovary from a white hen (W_3), mated to the black rooster, gave ordinary black, and black fetuses and chicks with white legs, in about equal numbers. In regard to the chicks from this hen described as ordinary black, some doubt exists as to whether the ventral light colored area described for normal black chicks was not lighter and greater in extent in all cases than in the normal chicks.

The white hen (W_3) carrying an ovary from a black hen (B_3), mated to the black rooster, gave uniformly spotted chicks, i. e., white chicks with black spots on the dorsal surface of the head, neck, wings, back or on the tail.

DISCUSSION OF RESULTS⁸

Color and Markings of Chicks

Owing to the uniform results from the controls (see Fig. 1), it may be assumed that the strains of chickens used breed true to color. Therefore any variations in the offspring from the operated hens were due to other influences.⁹

The fact that in all cases of the operated hens white or black, or spotted fetuses or chicks were produced (i. e., the offspring showed variations from the normal in color markings), shows:

1 *That the eggs from each of the operated hens were from the transplanted ovary.* Take hens B_3 and W_2 (Fig. 2). These hens were bred to the roosters of their color. Had some portion of their own ovary not been removed at the time of the operation¹⁰ (a remote possibility), and was functioning, then we would have expected solid color offspring like the controls. But

⁸ Size of hens, eggs, chicks, etc., will not be considered at this time.

⁹ The influence of the operation itself, as well as visual and other maternal impressions, including telegony and the completeness of the removal of the original ovarian tissue, will be rigidly tested in the new series of experiments now being started. In the present paper it must be remembered that these doubtful factors are ignored and the discussion is based entirely upon the results in the six hens herein reported.

¹⁰ Hen B_3 died as stated in the table, of indigestion. Postmortem examination revealed an ovary in all respects similar to that of a normal laying hen. This fact alone would seem to be all the evidence required to meet such objection, for should a minute portion of the ovary be overlooked at the time of operation, it is hardly to be expected that the Graafian follicles would increase in number.

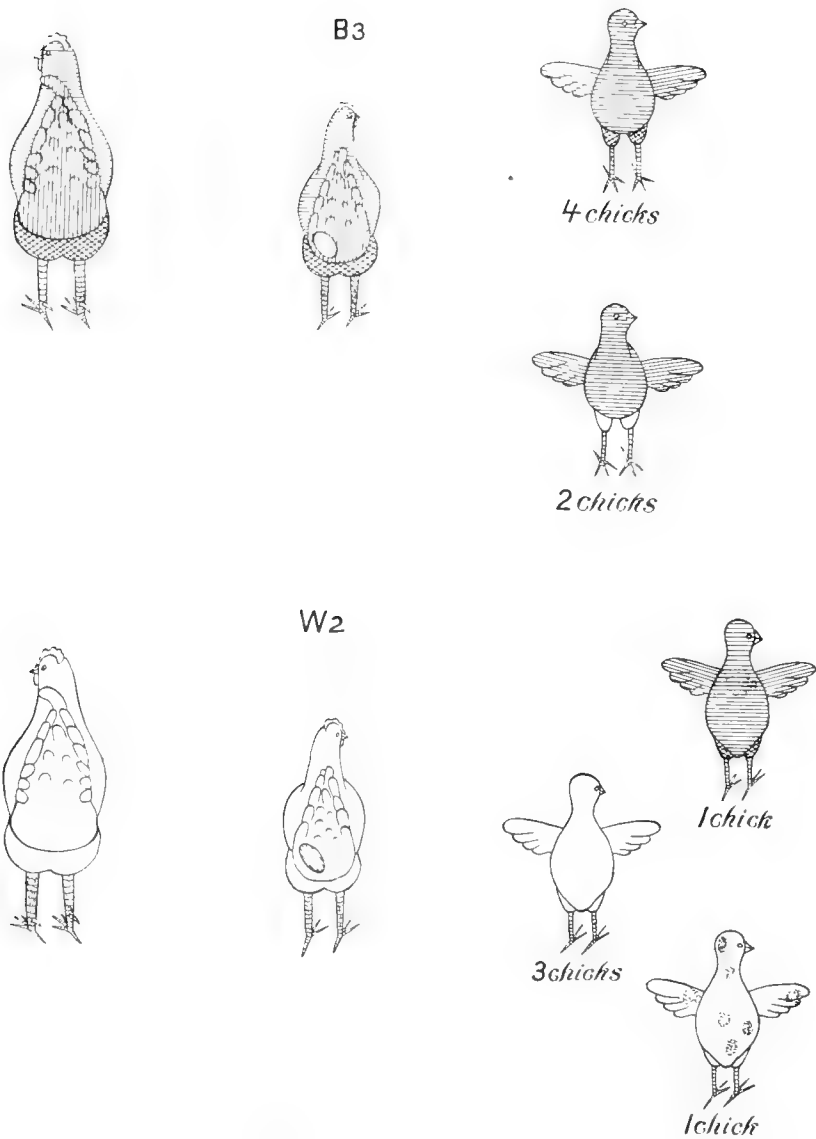


Fig. 2 Showing that transplanted ovaries function

such was not the case. In the offspring from B_3 , in which the male and foster mother were black, black predominated but white occurred. This must have come through the white ovary. In the offspring from W_2 , in which the male and foster mother were white, white was the predominating color but black occurred. The black therefore must have come through the black ovary.¹¹

If we accept the statement that in ordinary crossing of black and white breeds, the white is dominant, then we may assume that the same is not true for this kind of (female) crossing, or that the original color influence was more strongly preserved in the black than in the white ovary. From the constancy of the results in the above two hens, we may conclude that the ovaries transplanted into the other two hens, B_2 and W_3 , were the ones functioning during the laying season also.

2 *The foster mother exerted an influence on the color of the offspring.* Take hens B_2 and W_3 (Fig. 3). These hens were bred to the rooster of the opposite color, i. e., of the color of the transplanted ovary. Yet in the former the majority, and in the latter all of the offspring were spotted, i. e., white with black spots on the dorsal surfaces. In B_2 , the male and ovary were white and the foster mother black; in W_3 , the male and ovary were black and the foster mother white. In both cases white predominated in the offspring. It would seem therefore, if we leave the question of dominance out of account, that the foster influence of the white hen was stronger than of the black hen. If on the other hand we consider the foster influence equal in both cases, then we can explain the results as due to the dominance of the white in the male or ovary.

The Character of Feather Markings

In the white offspring, black spots occurred on the back, neck, head, shoulders, wings and tail in frequency in about the order given. In size they ranged from a few barbules on one feather to a patch of feathers the size of a dime. The larger spots were

¹¹ It is interesting to note the difference in the distribution of the ovarian color in these two cases.

observed on the back and head; the smallest on the wings. The markings of the feathers were not constant even in the same individuals. Some feathers were entirely black including the shaft, while others had scattered markings on the vanes involving only the barbs and barbules. In the black offspring, when white

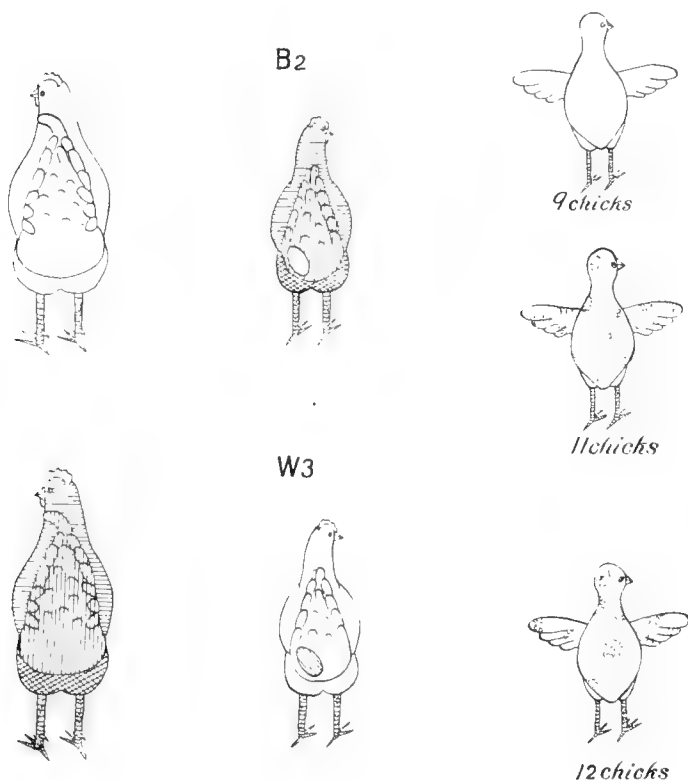


Fig. 3 Showing influence of foster mother.

occurred it did not appear as a spot but some part of the body, as the leg was solid white. No white feathers were found on the backs.

More data must be had on these points before definite conclusions can be drawn. Breeding the offspring may add to our knowledge along these lines. Similar experiments on chickens

and other fowls and on a number of different species of mammals are now being conducted. Other transmittable characters, as size, peculiarities of anatomical structure, etc., are being observed. Also experiments on the transplantation of the testicles are being made.

It will be of interest to determine if the adult ovaries, or if more embryonic ovaries will give concordant results when transplanted.

It seems that the qualities of the ovaries transplanted in these experiments may have been modified by the foster mother. Or the foster mother influence may have only been impressed after fertilization of the egg; or the influence may have been effective both before and after the egg was discharged from the ovary. A discussion of possible ways in which such influence might act would be unprofitable at this time. At present we are justified only in saying that a field appears to be open to attack with a reasonable hope of profit.

CONCLUSIONS

- 1 The ovaries transplanted in these chickens seemed to function in a normal manner.

- 2 The color characters of the resulting offspring appeared to be influenced by the foster mother.

Egg chart and table of results of controls and chickens with transplanted ovaries

FATHER	Black leghorn	White leghorn	White leghorn	White leghorn	Black leghorn	Black leghorn
MOTHER 1907	Black leghorn control	White leghorn control	Black leghorn, ovary from W_2	White leghorn, ovary from B_2	Black leghorn, ovary from W_3	White leghorn, ovary from B_3
February	B_1	W_1	B_2	W_2	B_3	W_3
1						
2	O lost					
3			O N F			
4	O N F		O N F			
5						
6	O N F					
7			O N F			
8	O N F					
9			O N F			
10	O N F					
11			O N F			
12						
13			O N F			
14						
15			O N F			
16			O S			
17						
18	O $\frac{4}{5}$ Bl. F		O F S			
19						
20	O $\frac{1}{4}$ Bl. F		O F S	O $\frac{4}{5}$ Wh. F	O $\frac{1}{2}$ Bl. F	
21	O $\frac{1}{1}$ Bl. F					
22			O F S		O F S	
23			O $\frac{1}{5}$ Sp. F			
24	O F S		O $\frac{1}{3}$ Sp. F	O $\frac{1}{2}$ Bl. F		
25			O Sp. Ck.		O $\frac{1}{3}$ Bl. F (Wh. legs)	
26	O F S		O $\frac{1}{4}$ Wh. F	O F S	O F S	
27	O $\frac{4}{5}$ Bl. F					O S
28			O $\frac{2}{3}$ Wh. F		O $\frac{1}{6}$ Bl. F	O S
March						
1	O F S	O F S	O $\frac{1}{3}$ Sp. F	O $\frac{2}{3}$ Sp. F	O F S	O S
2	O F S				O $\frac{1}{3}$ Bl. F (Wh. legs)	O S
3	O F S	O F S			O F S	O S
4		O F S	O S		O $\frac{1}{5}$ Bl. F	O $\frac{3}{4}$ Sp. F
5					O F S	
6	O S	O F S		O $\frac{4}{5}$ Wh. F	O $\frac{1}{3}$ Bl. F	O F S
7	O F S	O F S			O S	O $\frac{4}{5}$ Sp. F

Egg chart and table of results of controls and chickens with transplanted ovaries—Continued

FATHER	Black leghorn	White leghorn	White leghorn	White leghorn	Black leghorn	Black leghorn
MOTHER	Black leghorn control	White leghorn control	Black leghorn, ovary from W_2	White leghorn, ovary from B_2	Black leghorn, ovary from W_3	White leghorn, ovary from B_3
1907						
March	B_1	W_1	B_2	W_2	B_3	W_3
8	O F S					
9		O F S			O S	O 1/1 Sp. F
10	O 1/3 Bl. F				O S	O F S
11		O F S			O S	
12	O F S					O F S
13		O F S			O S	O S
14	O lost	O F S			O S	O S
15					O S	O F S
16	O F S	O F S				O F S
17			O F S		O S	
18	O S	O F S			O S	O S
19	O S	O F S	O F S			O S
20		O F S	O F S	O F S		O S
21	O S		O S			
22	O S	O S		O S		O S
23			O S			
24	O Bl. Ck	O S	O S			O S
25	O S	O S	O S			O S
26		O S	O S			
27	O S	O S	O S	O S		O S
28	O S	O S	O S			O S
29	O S	O S	O S		died	O S
30			O S	O S		O S
31	O Bl. Ck.	O Wh. Ck.	O 1/4 Wh. F			
April						
1	O Bl. Ck.	O Wh. Ck.	O S			O S
2			O 2/3 Sp. F			O S
3	O Bl. Ck.	O Wh. Ck.				
4		O Wh. Ck.		O Wh. Ck.		O Sp. Ck.
5			O 1/1 Sp. F	sick		
6	O 1/1 Bl. F	O Wh. Ck.	O Wh. Ck.			O Sp. Ck.
7						
8	O Bl. Ck.	O Wh. Ck.	O Wh. Ck.			O Sp. Ck.
9		O 1/1 Wh. F	O Sp. Ck.			O 1/1 Sp. F
10						
11	O S	O 3/4 Wh. F	O 1/2 Sp. F			O S
12						
13		O Wh. Ck.	O Wh. Ck.			

Egg chart and table of results of controls and chickens with transplanted ovaries—Continued

FATHER	Black leghorn	White leghorn	White leghorn	White leghorn	Black leghorn	Black leghorn
MOTHER	Black leghorn control	White leghorn control	Black leghorn, ovary from W_2	White leghorn, ovary from B_2	Black leghorn, ovary from W_3	White leghorn, ovary from B_3
1907						
April	B_1	W_1	B_2	W_2	B_3	W_3
14			O F S			
15		O $1/2$ Wh. F	O S			O $4/5$ Sp. F
16						
17			O Wh. Ck.			
18	O S					
19	O Bl. Ck.	O Wh. Ck.	O $1/1$ Sp. F			O $2/3$ Sp. F
20			O $1/3$ Wh. F			
21						
22	O F S	O Wh. Ck.	O $1/3$ Wh. F			O Sp. Ck.
23	O F S	O Wh. Ck.	O F S			O Sp. Ck.
24	O Bl. Ck.	O F S	O $1/3$ Sp. F			
25			O $1/3$ Sp. F			O $2/3$ Sp. F
26		O $1/1$ Wh. F				
27	O S	O S				
28		O F S				
29		O $1/1$ Wh. F	O S			O S
30		O S	O F S			
May						
1				O S		
2		O $1/2$ Wh. F	O S			
3						
4						
5		O $1/1$ Wh. F				
6		O S	O S			
7			O S			O S
8		O S				
9			O lost			
10			O F S			
11	O S	O S				
12						
13		O $4/5$ Wh. F				
14						
15	O S	O S	O S			O F S
16	O S		O S			
17			O S			O S
18	O S		O F S			
19						
20	O F S	O F S				

Egg chart and table of results of controls and chickens with transplanted ovaries—Continued

FATHER	Black leghorn	White leghorn	White leghorn	White leghorn	Black leghorn	Black leghorn
MOTHER	Black leghorn	White leghorn	Black leg-horn, ovary from W_2	White leg-horn, ovary from B_2	Black leg-horn, ovary from W_3	White leg-horn, ovary from B_3
1907	control	control				
May	B_1	W_1	B_2	W_2	B_3	W_3
21						O S
22	O S					
23			O S			
24		O S				
25						
26						
27			O S			
28			O S			O F S
29	O	O				
30			O			
31			O	O		
June						
1						
2						
3	O	O				
4						
5						
6	O	O	O			O
7						
8						
9						
10			O			O
11	O					
12		O				
13	O					
14						
15	O soft		O			
16	O soft					
17				O		
Days	Eggs 55	Eggs 52	Eggs 67	Eggs 13	Eggs 20	Eggs 45
135	Fetuses and chicks, 13	Fetuses and chicks, 18	Fetuses and chicks, 20	Fetuses and chicks, 5	Fetuses and chicks, 6	Fetuses and chicks, 12
	All black	All white	Spotted, 11 White, 9	White, 3 Black, 1 Spotted, 1	Black, 4 Black with white legs, 2	All spotted

Legend: O N F, egg, not fertile; O F S, egg, fertile, spoilt; O S, egg, spoilt, fertility not detected; 4/5, 1/4, 1/1, etc., relative size of fetus, 1/1 indicating full term, i. e., just ready to hatch; Bl. F, black

fetus; Wh. F, white fetus; Sp. F, spotted fetus; Wh. legs, white legs; Bl. Ck., black chick; Wh. Ck., white chick; Sp. Ck., spotted chick; O, egg, not tested.

Matings: Beginning February 14 to 17, hens B_1 , B_3 and W_3 , beginning February 28, were trod daily by the black Leghorn Rooster (BR) until March 5. From March 5 to March 29 they were not mated. From March 29 to date, they have been trod daily or every other day as before, i. e., by the black rooster.

Hens W_1 , W_2 and B_2 were trod as the above, only the white leghorn rooster (WR) was used.

March 29 hen B_3 died from indigestion. Postmortem examination showed her ovary to be in the normal position and in appearance normal for a laying hen both in size and structure. April 4, hen W_2 was found to be very badly infested with lice. Considerable difficulty was experienced in ridding her of them and she became emaciated.

Hen W_3 met with a serious accident early in January in which she suffered a compound fracture of the femur from which she recovered only in time to be added to the pens February 25.

The fowls have all been closely confined since February in quarters by no means ideal for the production of eggs for hatching.

The failure to hatch fertile eggs is attributed largely however to poor incubation.

HEREDITY, VARIATION AND EVOLUTION IN PROTOZOA

I THE FATE OF NEW STRUCTURAL CHARACTERS IN PARAME- CIUM, IN CONNECTION WITH THE PROBLEM OF THE INHERITANCE OF ACQUIRED CHARACTERS IN UNICELLU- LAR ORGANISMS

BY
H. S. JENNINGS

WITH 22 FIGURES

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INTRODUCTION

I Object of the Work

The investigations of which the first instalment is here presented are designed as the beginning of a study of the problems

of evolution in unicellular organisms. The question in the center of interest is: How do new inherited characteristics arise? To study this question a knowledge of the normal phenomena of variation and inheritance is required. Our first contributions will therefore deal with these normal phenomena, with incidental attacks on the main problem as opportunity presents. We shall take up inheritance, variation, specific differences, correlation, growth, regulation, selection, and related topics, dealing with them by experimental, observational and statistical methods. A large part of such a study, in the common infusorian *Paramecium*, is now complete. The present instalment deals with the definite and circumscribed problem of the fate of new structural characters.

2 *General Plan of the Investigation and Principles Guiding It*

In presenting the first instalment of an extensive series of investigations, it will be well to set forth in an introductory way the general considerations which have guided the work, together with its relations to previous investigations by the author. Though apparently a complete departure from the matters dealt with in most of my work up to this time, it is in reality a logical continuation of my previous work. The latter has lain hitherto in the field of the physiology of behavior and reactions. In this field I have endeavored to analyze and isolate, so far as possible, the various factors at work, keeping in the foreground of interest the problem of how the behavior happens to be so largely *adaptive*. It is possible to show that certain of the features of behavior—and precisely certain adaptive features—arise during the lifetime of the individual, by physiological processes which appear quite intelligible from a thoroughly causal standpoint. These are the processes known variously as the formation of habits; as learning; as modification by experience; as expressions of the readier resolution of physiological states after repetition, etc.

But in this field, as in all other parts of biology, we find many characteristics, and particularly many *adaptive* features, which have not arisen during the lifetime of the individual. Certain structures, certain processes, certain reactions, often highly adap-

tive in character, are found to be constituent parts of the organism, yet they have not arisen in the way mentioned, but are "inherited" from past generations. Such characteristics, in the field of behavior, are spoken of as reflexes, tropisms, instincts, etc.; they are often of a highly complex character.

Our next task is then to investigate the processes by which these characteristics have arisen. The problem is parallel—perhaps rather identical—with that which the student of structure sets himself when he asks how it happens that the animal possesses certain complex adaptive structures that are inherited from its progenitors. We cannot hold that complex characteristics can arise without any processes leading to them, unless we are prepared to abandon the scientific method. Where shall we look for the processes giving rise to characteristics that do not take origin in the lifetime of the present individual?

Clearly, there is but one possibility here. What we call the "individual life" is not the entire history of this mass of matter and energy that we call "an animal." It has existed for numberless ages in connection with other individuals, as "germ cell," or the like. Since the animal becomes modified and adapted in accordance with certain physiological laws, even in the brief span of its individual life, it is evident that the unmeasured ages of its previous existence could hardly pass without the occurrence of processes of modification. And it is only in this period that the processes could have occurred which have given it the complex inherited characteristics that it now has. We have then no alternative but to study the nature of these processes, if we wish to understand the origin of the characteristics under discussion.

Such a study of the processes by which organisms become modified in the life history of the race is of course as much a part of physiology as is the study of the processes of metabolism, and it must be pursued in the same spirit. Most of the existing science of physiology deals with the rapid processes taking place in the lifetime of the individual and in its "body." But of course the slower processes occurring in the germ material and resulting in modifications which become apparent in later generations are processes occurring in space and time, and open to objective experi-

mental investigation, exactly as are other physiological processes. There is the same reason to suppose them detectible by chemico-physical means as in the rest of physiology. There is indeed no reason for making any distinction in principle between these and the processes of movement or metabolism. The investigator in this field simply works on a part of the domain of physiology which has been mainly cultivated independently of the remainder of that science. In no way is the study of racial processes to be so much advanced as by considering this field, what it really is, a constituent part of physiology, and by attacking it from the same standpoints that have proved their worth in the rest of this science. Study of essentially this character is well under way in the work of the modern students of heredity—Bateson, De Vries, Davenport, Tower, Herbst, and others—though the point of departure has been in most cases not primarily physiological.

The *special* methods used—the *technique*—in a physiological investigation of racial processes will of course be extremely different from those of an investigation of metabolism or contractility; it is only in fundamentals that the method of attack must be the same. Every problem requires its own technique. In the study of racial processes we have to deal with certain problems and phenomena which have as a rule not been looked at from a physiological point of view. They are nevertheless physiological matters, and need restatement in physiological terms. Let us attempt this:

Evolution, from this standpoint, is a general name for the physiological processes which result in change of characteristics from generation to generation. The physiological study of evolution is the objective and experimental investigation of these processes.

Adaptiveness, purposiveness, teleology, etc., are concepts based on the observed phenomena that the characteristics of organisms are largely of such a nature as to maintain the processes which we call life, and thus keep the organisms in existence. From a purely physiological point of view the teleological problem is essentially this: How does it happen that combinations of such complexity

of structure and action can continue to exist? Or to put the question in a way that leads directly toward investigation: What processes lead to the production of lasting combinations, of such complexity of structure and action as are found in organisms?

In considering this question, we are struck by the evident fact that certain combinations of the various factors making up the universe *are more lasting than others*. Two constituents (as gold and oxygen) come in contact; they do not unite, and the combination constituted by their juxtaposition is quickly dissolved by the incidence of other forces. Two other constituents (as iron and oxygen) come into contact; they unite, and the combination resulting from their juxtaposition is a relatively lasting one. Such varying permanence of different combinations is seen in every field, but it is particularly striking among such complex bodies as go to make up organisms. Here the persistence of certain combinations and the evanescence of others is commonly spoken of as *selection*. The combinations which persist are said to be *selected*. The term is undoubtedly, for certain reasons, an unfortunate one.

In the study of organisms, as we have seen, one great class of problems lies in the question, How can such complex combinations as organisms be *lasting*? Now, the study of what combinations are lasting is precisely the study of so-called selection, and so it happens that in the investigation of the processes by which organisms have acquired their characteristics, the study of selection necessarily plays a very large part.

Selection has often been looked at from an extremely narrow loophole, so that only a small part of it has been seen. In a common case, only the fact that certain *individual animals* are more lasting than others is taken into account; on this selection from among individuals attempts have been made to base an entire theory of organic evolution. It would seem incredible that anyone should suppose the principle of selection to be limited in its operation to this one class of combinations, did not history show that such views have been held. Selection is merely a name for certain aspects of the way the world process takes place. The

*This formulation of the problem we owe essentially to Jensen ('07), whose valuable paper cannot be too strongly recommended to those who wish to view such problems from a physiological standpoint.

greater permanence of certain combinations and activities is evident everywhere outside the limits of organisms, while within the system making up the individual organism there are conditions which require the prevalence of this principle of operation, on a large scale. To selection, or the greater permanence of certain combinations, *within* the organism, we must look for an understanding of many of the most important problems of biology, and particularly of those having to do with adaptation. The study of the internal adaptations of organisms might indeed be defined as the search for those combinations of structure and activity that are most lasting. The study of the laws in accordance with which certain combinations are lasting, while others are fleeting, must become one of the main lines of investigation. The pioneer work of Roux ('81) in this line was most promising, and has been followed up to a certain extent; but thorough experimental investigations along such lines are what is needed. In the meantime, the relative permanence of those combinations which we call individuals must remain one of the chief objects of study. As Kellogg ('07) has well noticed, we have few, if any, cases even of this, that are clearly and accurately observed and analyzed.

All together, it is clear that a study of the processes which result in the complex "adapted" organism must be largely a study of the relative permanence of different combinations—a study of selection. This of course requires a study of the chemico-physical laws in accordance with which the processes are brought about, and in accordance with which some of their products are more lasting than others. It is in many respects unfortunate for an understanding of this line of work that it has received the figurative and anthropomorphic name of *selection*. When we speak merely of the relative permanence of different combinations (whether these combinations are individuals, processes, or chemical compounds), we call up no associations foreign to the matter in hand, and thus run no risk of arousing misconceptions and prejudice due to such associations.

In studying the racial processes, that have resulted in giving the organism its "hereditary" properties, we meet one great difficulty. We cannot reproduce the long series of conditions which

have acted upon the organism when it lived in connection with individuals of past generations. We cannot hope, then, to study the precise processes which have given rise to the particular combination of characteristics which we find in *Paramecium* or the dog or in any particular existing organism. All we can hope to do is to study similar processes in progress, controlling and analyzing them experimentally, till we work out the laws and principles of their action. By application of what we thus gain to the results of processes past, we may hope to reach an understanding of how organisms have arisen.

3 Place of the Present Investigations in this Plan

In taking up a study of these racial processes, we must first learn as accurately as possible what occurs in the passage from one generation to another; what resemblances and differences are normally found between members of succeeding generations, and the like. In other words, we must have a knowledge of the normal phenomena of heredity and variation, such as is now being acquired on a large scale in higher animals. When this is obtained we may proceed to attempt to modify experimentally the processes and their results—thus approaching the central problem: How do inherited modifications arise?

In such work, the relations found in the simplest organisms deserve investigation. Here we have reproduction taking place rapidly (a generation or more a day) and in the simplest forms. I have therefore undertaken a study of the physiology of racial processes in the Protozoa. Bearing more or less directly on this matter we have already a large amount of most valuable work, such as that by Maupas, Hertwig, Schaudinn, Calkins, Woodruff, Enriques, and others. I have approached the matter however from a different standpoint, setting the problems of inheritance and variation definitely in the center of interest. This results in somewhat different methods of attacking the subject.

ASSUMED DIFFERENCE IN HEREDITY BETWEEN UNICELLULAR AND
MULTICELLULAR ANIMALS—THE “INHERITANCE OF ACQUIRED
CHARACTERS”

It is often said, and it seems to be generally assumed, that unicellular animals differ fundamentally from multicellular ones in heredity.* In the Protozoa there is no separation into cells which normally die after a certain period (“somatic” or “body” cells), and cells which continue to live and multiply (“germ” cells). The parent produces progeny by simply dividing, so that parents and progeny are identical.

This seems to simplify extremely the problem of heredity, or indeed to remove everything problematical from the subject. Parents and progeny must be *alike*, it is said, because they are *the same*. In particular it is commonly held that this removes from the Protozoa all difficulty as to the “inheritance of acquired characters”—characters added during the lifetime of the individual and due to environmental action, experience, use, accident, or the like. Such characters are in multicellular organisms often called somatic, as distinguished from germinal, and such somatic characters are commonly held not to be inherited. Where there is no such distinction between soma and germ, it would seem clear that there can be no distinction between somatic and germinal characteristics.

To this difference in heredity between Protozoa and Metazoa much importance has been attached. If the difference really exists, the Protozoa are much more plastic in evolution than are the Metazoa; through the inheritance of the effects of experience, use and environment, the Protozoa must permit of the ready and rapid production of varied and adapted types. This point has been emphasized by many writers. For example, in attempting to account for the great diversities of organization and action found among animals Whitman ('99) writes as follows:

“In primitive organisms multiplying by simple fission, structural modifications acquired during the lifetime of the individual

*I use the word “heredity” merely as a brief and convenient term for “the resemblance between parents and progeny,” without implying any underlying entity, and without prejudice as to the grounds of this resemblance.

would be carried right on from generation to generation, and hence structural foundations for a whole animal world such as we now see could be laid in a relatively short period as compared with the time necessary to advance organization in forms limited to reproduction by germs. In fact the fundamentals could all be established within the realm of the unicellular Protozoa" (p. 307).

In my book on the Behavior of Lower Organisms, I expressed similar ideas, with particular reference to the inheritance of ways of behaving:

"In the unicellular organisms there seems to be nothing in the way of this inheritance by the offspring of the reaction-methods acquired by the parent. There is no distinction between the germ cells and body cells in these organisms: all acquirements pertain to the reproductive cells. Through reproduction by division the offspring *are* the parents, merely subdivided, and there is no evident reason why they should not retain the characteristics of the parents, however these characteristics were attained. If this is the real state of the case, then in unicellular organisms the life of the race is a direct continuation of the life of the individuals, and any acquirements made by the individuals are preserved to the race" (Jennings '06, p. 320).

Now, if this difference between unicellular and multicellular organisms actually exists, it is evidently of the highest interest and importance. Yet there have been no investigations of the matter to see if there really is such a difference. Our first task is then to examine the phenomena from this standpoint; attempting to determine whether characteristics acquired during the lifetime of the individual* are inherited by the progeny. At the same time, we shall keep in mind the broader aspects of our problem, endeavoring to work out in general the relation of reproduction in the Protozoa to heredity.

*I use for convenience the term "individual," as commonly employed, to signify in the Protozoa the separate free cells. I have no wish thereby to take any stand on Calkins' contention that the entire cycle of cells derived from a conjugating pair corresponds to the individual of the Metazoan (Calkins '06). The present paper deals with certain existing phenomena, which are not altered by the views one may hold on this point. The relation of conjugation to heredity is to be taken up in a later communication.

THE FATE OF NEW STRUCTURAL CHARACTERS ("ACQUIRED CHARACTERS")

As we have just seen, it is commonly held that "acquired characters" are inherited in the Protozoa, though not in the Metazoa. Do experiment and observation show that this is true? Does the separation of germ and body cells make a fundamental difference in heredity?

1 Localized and Unlocalized Characteristics

In dealing with new or "acquired" characters, it is well to distinguish two classes. On the one hand are those characters (mainly structural), that are localized in a definite part of the body, as cilia, setæ, a mouth, etc. On the other hand there are characters that affect the organism as a whole; such are acclimatization or other general modifications due to heat, cold, chemical agents, etc.; size, method of growth, and the like. The inheritance of the latter class of characteristics, however acquired, presents much less apparent difficulty than does the inheritance of the former. The importance of this distinction between localized and unlocalized characteristics, in investigations of heredity, has often been emphasized. Weismann has repeatedly demanded as proof of the inheritance of somatogenic characters in Metazoa a demonstration of "the transmission of changes of single definite parts of the parents to the corresponding parts of the progeny;" of the inheritance of "definite parts or localized functions." It is clear that a somewhat different problem is involved in the two classes of cases. We shall take up first localized characters in the Protozoa.

2 Typical Examples of Inheritance and its Problems in Protozoa

To appreciate the problem of the inheritance of localized characters, we will look at one or two simple cases in the Protozoa; these will serve to bring the whole problem of inheritance in these animals to a point.

Paramecium (Fig. 1) has a blunt anterior end and a pointed posterior end. How does it happen that after fission similar

features are found in the progeny? The animal has in the anterior half an oral groove; near its middle a mouth; near the aboral side two contractile vacuoles. How does it happen that the progeny have similar structures? If one of these structures should become modified in the parent, would this modification appear in the progeny?

For a more complex case, we have in *Oxytricha* (Fig. 2), a definite, typical distribution of the organs of locomotion. There are, for example, regularly five large setæ in a row near the pos-

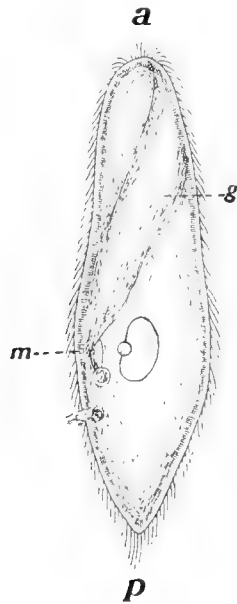


Fig. 1 *Paramecium*, to illustrate the problems of inheritance in Protozoa. By mere transverse fission the blunt, grooved anterior end *a* would be left with only one individual, the sharp posterior end (*p*) with another. *m*, mouth; *g*, oral groove.

terior end (*S*, Fig. 2). In other infusoria, related to this one, these setæ appear in different form, number or arrangement. How does it happen that after fission the progeny have setæ of the same size, structure, arrangement and position as did the parent? If the parent loses one of these setæ, will the reduced number appear

in the progeny? Similar questions must be asked for each of the organs of locomotion and other structures, seen in Fig. 2.

These questions regarding details show that we do not after all gain much for understanding inheritance in Protozoa by such statements as that "parent and progeny are the same and so must be alike." For in simple transverse fission of *Paramecium* there is no reason that is at once apparent, why the anterior product should have at its posterior end a point, as its parent had, nor why the posterior product should have a blunt anterior end with a

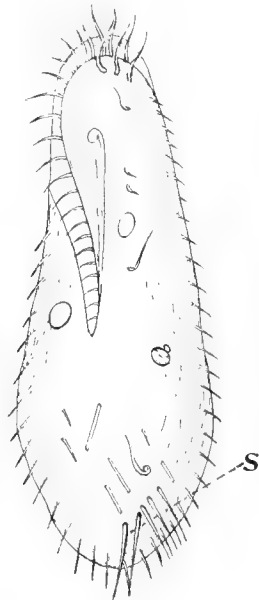


Fig. 2. *Oxytricha fallax*. Mere transverse fission would leave the five large setæ *s* with only one of the resulting individuals.

groove along one side; these are not simply passed on, ready made, to the progeny. Again, the simple transverse fission of *Oxytricha* does not account in the least for the fact that the anterior product of division has the row of five setæ at its posterior end. The five setæ might be transmitted directly to the posterior daughter-infusorian, but the anterior individual would naturally be left quite without such structures. Indeed, by repeated mere divisions

(even if followed by increase in size), progeny would after a time be produced that would have little resemblance to the parent.

Thus it is evident that even in the Protozoa heredity is not a mere result of subdivision. The question returns with force: How does it happen that the localized structures of the progeny are the same as those of the parent? And *are* they the same in all cases? Are they the same when the characteristics of the parent have become changed during its lifetime as an individual?

We shall take up first the simplest and most marked characteristics—new appendages, spines and the like; marked changes in the form of parts of the body; all sorts of things that might be characterized as mutations, abnormalities, monstrosities, etc. We shall deal at the same time with mutilations.

THE FATE OF NEW LOCALIZED STRUCTURES IN PARAMECIUM, WITH
OBSERVATIONS ON GROWTH AND REGULATION OF FORM IN
THIS INFUSORIAN

By examination of dense cultures of *Paramecium** many individuals were found which differed in certain respects from the usual form or structure. Some had a short, truncate anterior end; others a blunt or truncate posterior end in place of the sharp tip; others were crooked or otherwise modified in form; others showed angles, teeth or spines on various parts of the body. Many of these were isolated and allowed to reproduce under observation, so as to follow the fate of the peculiarity in question.

The method of isolation and culture was essentially that described by Calkins ('02). The individuals were placed separately in the concavities of hollow-ground glass slides, in three or four drops of hay infusion, which was changed either every day or every two days. The animals were examined once or twice a day.

1 History of a Large New Appendage in Paramecium

I shall first describe in detail a typical case of a new structure; an individual that bore on its body a spine (Fig. 3). This case is particularly instructive because the origin of the peculiarity

*The animals studied had the characteristics usually attributed to *Paramecium caudatum*. The question of distinguishing species will be taken up in later parts of this general investigation.

was observed, and its history followed for many generations. The observations on this structure likewise give certain important results as to the method of growth in *Paramecium*.

First generation. The ancestor of the race we are to study was a crooked individual (Fig. 3, *a*), found in a culture containing many specimens, where food was getting scarce. I have called this individual *a*; we shall use this designation for the race as a whole, appending certain exponents to indicate the various members of the different generations. The anterior individual resulting from fission will be designated by the exponent (¹), the posterior individual by the exponent (²).

The original individual *a* was bent just in front of its middle at practically a right angle (Fig. 3, *a*). It was isolated at 2.50 p.m., May 2, 1907.

Second generation. The first division, during the night of May 2, showed that the crookedness was not to be inherited, though it had its effects on the progeny. The animal divided transversely, posterior to the bend in its body. The posterior product (*a*²) was normal in all respects, so that it need not concern us further. The anterior product (*a*¹) was of about the form that would be expected from dividing *a* behind the bend in its body, save that the posterior end had become still more irregular. This end was broad and truncate; nearly triangular when seen from the rear; it extended backward at two of the angles as two pronounced points (Fig. 3, ²).

Shortly after division the daughter individual *a*¹ changed shape greatly; the posterior end budded out a new structure of nearly the normal shape for the posterior half of the body (Fig. 3, ³). But this new part formed an angle with the anterior half, so that the body of this individual was again crooked. At the same time the anterior end extended a little. The two teeth remained near the middle of the body, the larger one having been carried back a little, so that it was a little behind the smaller one.

Third generation. At the next division (forenoon, May 4) the constriction appeared between the two tooth-like projections, and the plane of division was oblique (Fig. 3, ³). Thus the smaller one of the two projections was at the posterior end of the

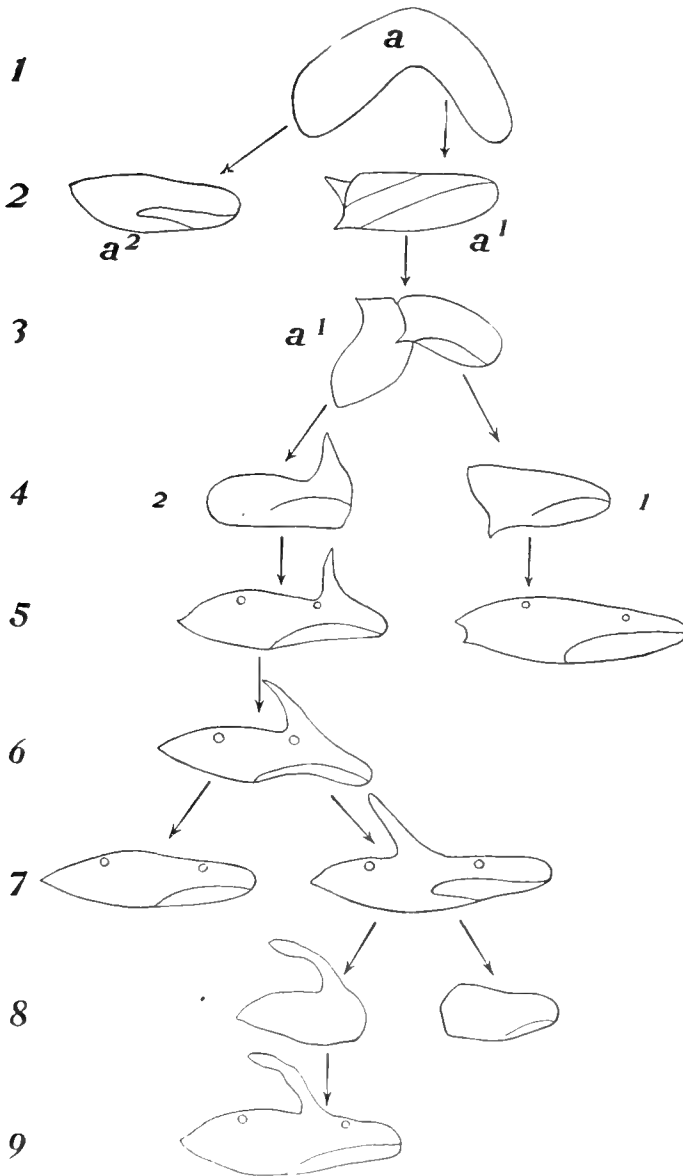


Fig. 3 Transformations in the race *a* during the first four generations. The anterior end is to the right. *a*, the original crooked individual (first generation). *a*¹, *a*², the anterior and posterior products of its fission. At 3 the individual *a*¹ has grown and is dividing, giving the individual 1 (anterior) and 2 (posterior). The arrows show the origin and transformations of each individual. For details, see text.

anterior product $a^{1.1}$, while the larger projection was at the anterior end of the posterior individual $a^{1.2}$. In the period just before and after the separation of the two parts (which occurred at 10.55) this larger projection grew rapidly still larger, longer and sharper, as if it were being pushed out under pressure. Immediately after division the posterior product $a^{1.2}$ had the form shown in Fig. 3, ⁴, the projecting spine being as long as the body was thick, and situated on the aboral side, nearly at the anterior end.

Now this posterior individual $a^{1.2}$ began to grow rapidly. Growth was most rapid at the anterior tip; this pushed out so as to leave the spine at some distance from the anterior end. The spine itself became still longer and stouter. At the same time the entire body increased in length, the growth seeming most rapid at the anterior end and decreasing toward the rear. Twenty minutes after division the posterior individual $a^{1.2}$ had the form shown in Fig. 3, ⁵.

The change of form now continued much more slowly, so that at the end of four hours the shape was that shown in Fig. 3, ⁶.

In the anterior individual ($a^{1.1}$) a parallel process of growth occurred; the anterior part of the body pushed out rapidly, while the posterior part merely changed shape a certain amount. The small projection was thus left near the posterior end, on the oral surface (Fig. 3, ⁵).

Thus we have now on each of these individuals a definite new structure, the origin of which we know, while the animals are quite normal in other respects. The new structures have arisen during the reproductive processes—at a period comparable, if there is any such in the life of the infusorian, to the germ cell period, just before development begins, in a Metazoan. Tower ('06) found that in certain Metazoa changes wrought in the organism at this stage of its life give rise to permanent inherited modifications, though environmental effects at other stages are not inherited. We have then perhaps as favorable a case for studying the transmission of a suddenly produced new structure as we could expect to find in the Protozoa.

We shall here follow only the history of the large anterior spine, in $a^{1.2}$ (Fig. 3, ⁶), taking up later the fate of the short tooth in $a^{1.1}$.

Fourth generation. We left the individual with the long anterior spine in the condition shown in Fig. 3, ⁶. At the next fission (night of May 4) the spine remained with the anterior product $a^{1 \cdot 2 \cdot 1}$, while the posterior product $a^{1 \cdot 2 \cdot 2}$ was a typical individual without a spine. In this fourth generation, since the division had taken place at the middle and there was subsequent outgrowth of the anterior tip, the spine was left behind the middle of the new individual (Fig. 3, ⁷). The spine itself had become still longer and more slender. In structure it was a tube of ectosarc enclosing a narrow canal filled with endosarc. It was flexible, bending readily when it came in contact with obstacles, but it did not show active movements.

Fifth generation. At the next division (noon, May 6) the plane of division lay just in front of the base of the spine, so that the latter went to the posterior individual ($a^{1 \cdot 2 \cdot 1 \cdot 2}$), and was situated at its anterior end (Fig. 3, ⁸). The other (anterior) individual ($a^{1 \cdot 2 \cdot 1 \cdot 1}$) was normal, as usual. In the process of growth, consisting largely in the pushing out of the anterior end, the spine came to lie farther back than at first, so that in the adult infusorian it was a little in front of the middle (Fig. 3, ⁹). The spine had become slightly enlarged at its tip, and bent to the right at about its middle.

Sixth generation. The plane of the next division (night of May 6) passed just behind the spine, so that the latter was now left on the anterior specimen, $a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1}$, while the posterior specimen was normal. The spine was now bent near the base, so as to extend backward parallel with the body axis (Fig. 4, ⁶).

Seventh generation. At the next division (night of May 7), the spine of course went to the posterior individual, $a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2}$ (Fig. 4, ⁷). It was situated a trifle in front of the middle of the body. The spine was now long and curved downward and backward over the right side of the animal. Its base was much broader than before, and a shorter spine had pushed out forward from the angle between the base of the spine and its main body.

Eighth generation. At the next division (night of May 8), the spine went to the anterior individual ($a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2 \cdot 1}$) and was situated very nearly at its posterior end, though a little displaced toward

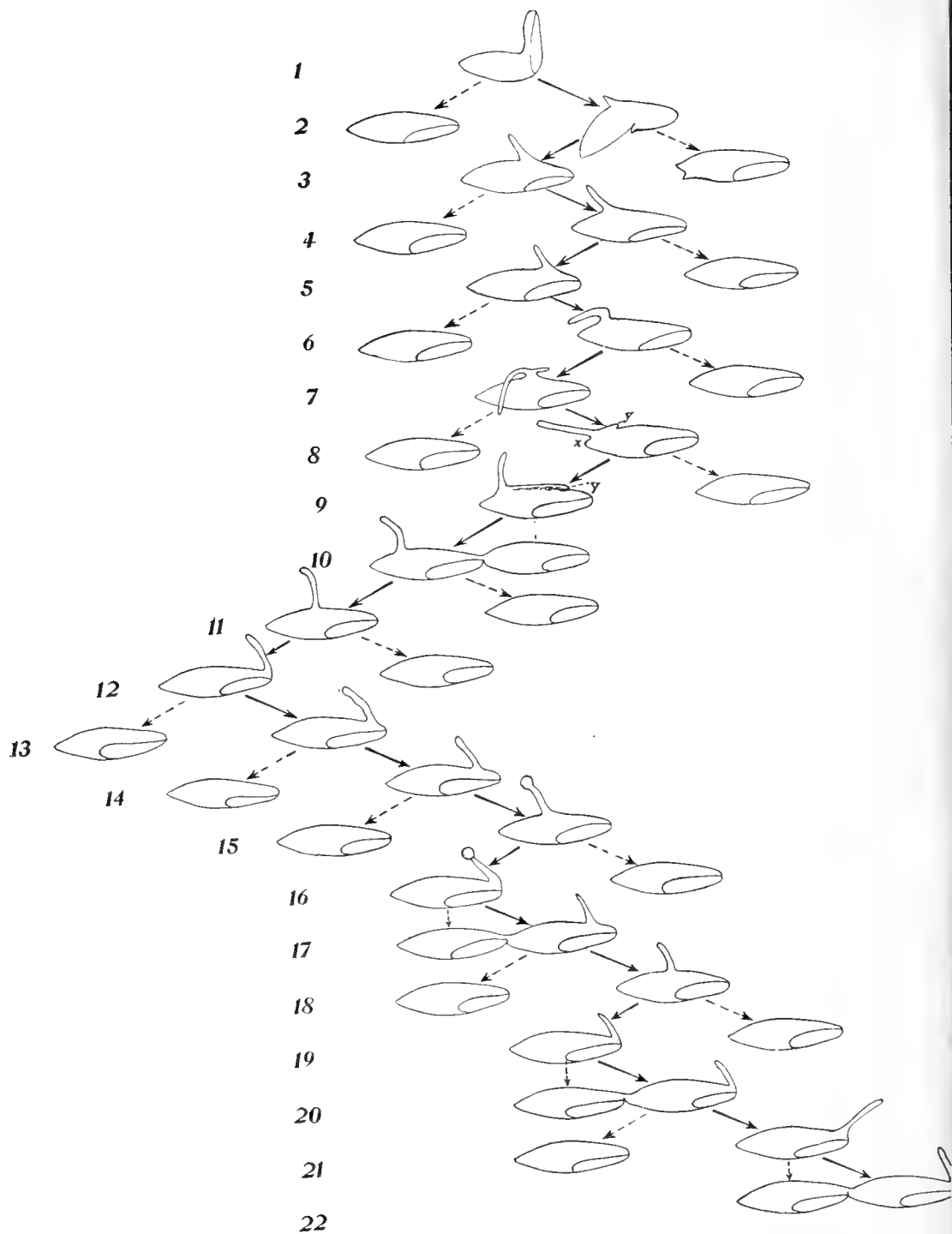


Fig. 4 Diagram of the history of the race *a*, bearing the spine, for the entire twenty-two generations. The anterior end and anterior individual are throughout to the right. The numbers at the left indicate the generations, counting the original crooked specimen as the first. The arrows show the lines of descent. Only the fission of the individual bearing the spine is followed out in each generation. For details, see text.

the aboral side. Its base had become broad and low, extending between x and y , Fig. 4, ⁸. There is reason to think that it actually extended back of x , to the posterior end. It would naturally be carried back in the backward growth of the posterior tip, but owing to the abrupt point naturally found here, there is nothing to mark the end of the base, as there is at y . The anterior point (at y) had dwindled to a mere knob, while the main spine trailed behind, half the length of the body.

Ninth generation. At the next division (night of May 9), the spine passed, as was to be expected, to the posterior individual $a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2}$. Certain interesting changes have taken place in the position and structure of the spine, which throw light on the processes of growth, and which have important consequences for succeeding generations. The free part of the spine is still very near the posterior end, and stands again at right angles to the body (Fig. 4, ⁹). The broad base of the appendage (x - y , Fig. 4, ⁸) has been still farther drawn out in the processes of growth, so that it extends forward almost to the anterior end (to the point y , Fig. 4, ⁹). Posteriorly its end is not evident, but it doubtless reaches to the posterior tip. Thus the base of the spine now extends nearly the entire length of the body, so that it must be cut by the next fission plane.

It will be observed that up to this time the spine has regularly alternated between the anterior and posterior individuals in the successive generations. This is indicated in the designation employed ($a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2}$), the exponent (¹) indicating in each case the anterior product of fission, the exponent (²), the posterior product. When situated on the anterior individual the spine lies back of the middle of the body (see Fig. 4, ^{4,6,8,10}, etc.) When on the posterior individual it has always lain in front of the middle of the body (see Fig. 4, ^{3,5,7}), till in the present generation (Fig. 4, ⁹). These changes in position are due to the growth occurring after fission; they give us a means of analyzing this growth—a matter to be taken up later.

Tenth generation. At the next fission (May 10, day) the free portion of the spine went again to the posterior individual, thus breaking the regular alternation which has prevailed up to this

time. The individual bearing the spine is therefore $a^{1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2 \cdot 1 \cdot 2 \cdot 2}$. The effect of the ridge forming originally the base of the spine (x - y , Fig. 4, ⁸) is shown in the fact that the two individuals did not separate, as usually happens; they remained connected by a sort of bridge passing along the aboral surface (Fig. 4, ¹⁰). Evidently the substance formed by the extended base of the spine is not so easily cut by the processes of fission as are the other parts of the body; it therefore forms the bridge. The two individuals thus connected did not move in unison; there was much pulling, bending and twisting of the slender connecting bridge, so that the latter appeared likely to break. In the course of time this happened; the two individuals separated some time during the next night, before the next fission occurred.

As will appear in the sequel, this tendency to remain connected even after the adult condition is reached persisted in the progeny of these individuals for many generations. We have therefore something resembling the inheritance of a new characteristic. This matter will be taken up in a separate section.

The spine still remained near the posterior end of the individual, though not so near it as in the previous generation. The posterior tip has pushed backward from the spine, in the growth that takes place after division. It carries with it some portion of the base of the spine, just as happens in front.

Eleventh generation. Again the spine went to the posterior individual (night of May 10). As would be expected, the spine is now further forward; it is again nearly straight and at right angles to the body (Fig. 4, ¹¹).

Twelfth and thirteenth generations. During the night of May 11 there were two fissions, giving three specimens of the normal form, and one with the spine. It appears clear that at the first of these two divisions the plane of fission was just in front of the spine, so that the latter was left almost squarely on the anterior tip of the posterior individual; here it remained till the next division. This time of course the spine went to the anterior individual, still remaining almost exactly at the anterior end. In its outgrowth the anterior tip has carried the spine with it, owing to the fact that the latter was almost at the very end. The individual

bearing the spine in the thirteenth generation is therefore to be designated $a^{1.2.1.2.1.2.1.2.2.2.1}$.

Fourteenth and fifteenth generations. During the night of May 12 there were again two divisions, giving three normal individuals and one with the spine. The spine is now situated at about the middle of the body (Fig. 4, ¹⁵). The only way this result can have been reached is as follows: The spine went to the anterior individual in both of these divisions, and in the growth processes after each division it moved backward about one-fourth the length of the body (or rather, the anterior tip grew forward that amount). The individual of the fifteenth generation is therefore $a^{1.2.1.2.1.2.1.2.2.2.1.1}$.

The spine now bears a ball at its tip (Fig. 4, ¹⁵). This is due to the fact that at the time of fission some of the endosarc is squeezed out through the tube of ectosarc, thus forming the ball. This indicates that at the time of fission, or in the period of rapid growth just following it, the internal contents must be under much pressure.

Sixteenth generation. The plane of fission (night of May 13) passed just in front of the base of the spine, leaving the latter at the anterior tip of the posterior individual (Fig. 4, ¹⁶). Again it failed to be displaced backward in the growth following fission. The ball at the end of the appendage was gradually constricted off from the tip, becoming completely separated at 10.15, May 14.

Seventeenth and eighteenth generations. During the night of May 14 the animal again divided twice. The method of division is shown clearly by the fact that the three individuals without the spine remained connected in a chain, only the animal bearing the spine being free. The spine went to the anterior individual in both fissions, being displaced backward about one-fourth the body length in each growth period.

Nineteenth generation. In the next division (night of May 15), the spine went to the posterior individual, being borne again at the anterior tip (Fig. 4, ¹⁹).

Twentieth and twenty-first generations. During the night of May 16 there were two generations, the spine going to the anterior individual in each case. This is demonstrated by the fact that the three individuals without the spine have remained united in a chain

while the spined animal is free. *The spine is still at the anterior tip*; it has not moved backward for two generations.

This individual did not divide for more than twenty-four hours, and during its lifetime the spine became a little shorter. The animal now *used the spine* almost continually. It placed the tip of the spine against the bottom of the vessel or against any other surface, then ran along the surface, keeping the tip of the spine in contact, while currents of water passed down the oral groove (Fig. 5). This use of the spine is of course incidental to the common habit of these animals, of placing one side of the body against a surface and running along it. But this is the first generation in

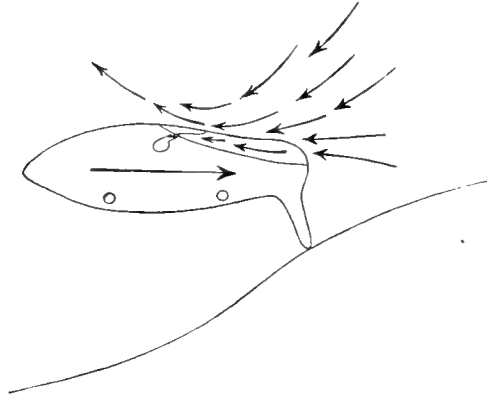


Fig. 5 Use of the spine by the individual of the twenty-first generation. The tip of the spine is pressed against a surface and the animal runs along it, in the direction indicated by the large internal arrow, while the currents of water down the oral groove to the mouth are indicated by the small arrows.

which such a use of the appendage occurred. This, taken with the fact that the appendage seemed to be gaining a permanent position at the anterior tip suggested possible interesting developments in the future.

Twenty-second generation. The spine again remained at the anterior tip. The division (afternoon of May 18) was at first not complete (Fig. 4, ²²), the animals remaining connected for more than twenty-four hours.

On the morning of May 20, the two had separated, but had not divided farther. Both were swollen and opaque; they were evi-

dently in an unhealthy condition. Investigation showed that the wrong sort of bacteria had multiplied in the culture fluid last made, making it opaque and sirupy. All the specimens (for various other experiments) that had been placed in this fluid were unhealthy or dying. This multiplication of injurious bacteria in culture fluid made in the usual way, is a not uncommon and most disastrous occurrence. To it we shall return in another connection.

The two sister individuals (one with the spine, the other without) were transferred to clean water, and later to new culture fluid. They were still living May 21, three days after the last preceding fission. But on the morning of May 22 I found, to my great regret, that the individual with the spine had died. Its sister recovered and propagated the race for many generations, of which we shall have to speak in our account of the hereditary tendency to remain connected after fission.

The last individual bearing the spine was designated $a^{1.2.1.2.1.2.1.2.2.2.1.1.1.2.1.1.2.1.1.1}$. These exponents show to which individual the spine passed at each division—⁽¹⁾ indicating the anterior individual, ⁽²⁾ the posterior one. The spine was traced through twenty-one generations (the first generation not having the spine). Fig. 4 gives a diagram of the entire history of this structure.

In this history of a localized new structure for twenty-one generations, certain general relations appear, which we will here set forth, though a full discussion of their significance will be reserved till other cases have been considered.

2 General Relations and Processes Shown in the History of the New Structure

1 The new structure was transmitted in each generation to but one individual. Thus, in the sixth generation there were thirty-two individuals, with but one bearing the spine (see Fig. 6); in the eleventh generation, out of 1024 individuals, but one had the spine; in the twenty-second generation the spine was found on but one individual out of 2,097,152.

Furthermore, the spine occupied a definite place in the series of individuals produced. As we have seen, and shall see farther,

sometimes *Paramecia* do not completely separate after division, but remain united in chains. If we conceive of all the individuals of each generation as thus forming a chain, each being in the position that the method of transverse fission gives it, then on such

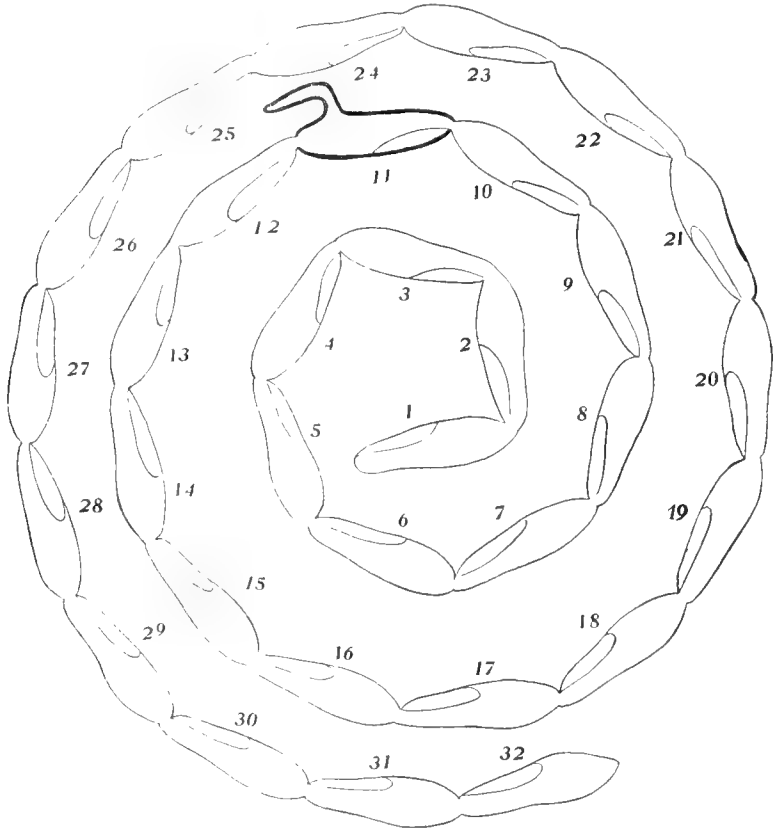


Fig. 6 Diagram showing the position and relations of the spine of *a* in the sixth generation, when thirty-two individuals are present. The individuals are conceived to have remained united, in the positions given them by the successive fissions. The spine would be found on the eleventh individual from the anterior end of the chain (drawn with a heavy outline).

a chain we would find but one spine, having a certain definite position. Thus, in the sixth generation, where thirty-two individuals were present, the spine would have been situated as shown in Fig. 6, on the eleventh individual from the anterior end of the

series. In the ninth generation, after eight regular alternations from the anterior individual to the posterior one and back, in the fissions, we should have a chain of 256 individuals, with the spine on the 171st individual counting from the posterior end of the chain. In the twenty-second (final) generation, the chain would be 2,097,152 individuals long, and would bear but a single spine situated on the individual numbered 1,393,592 from the posterior end.* Such a chain would be about 419 meters long, with the spine about 278 meters from the posterior end.†

Thus though the new structure is transmitted it is not multiplied, and there is no tendency to produce a race with this characteristic. There is evidently a fundamental difference between on the one hand this simple handing on of a localized structure to one of the new individuals, and on the other hand, the reappearance of the localized structure in all or many of the individuals resulting from fission. The difference is in some respects similar to that between "somatic" and "germinal" characters in Metazoa. This point we shall take up later.

2 The position of such a structure on the body of the individual is not permanent and the same in succeeding generations. The same structure is found in one generation at the anterior end, in another at the posterior end; now at the middle; now in some intermediate position. At first the structure alternated regularly between a position nearer the posterior end, and one nearer the anterior end; later its wanderings were wider.

These fluctuations of position are due mainly to the processes of growth following fission. These processes will be analyzed quantitatively in later communications; here we see merely the main facts in a general way. After fission the entire body lengthens, both ends pushing out rapidly. The anterior tip pushes out somewhat more than the posterior one. In consequence, a structure located, just after fission, near the anterior end (Fig. 3, ⁸) is

*The rule for finding which individual of a given generation would bear the appendage is as follows: If in a certain generation the number of individuals posterior to the one bearing the spine is x , then in the next generation, if the spine goes to the posterior product the number posterior to the spined individual will be $2x$; if the spine goes to the anterior product, the number will be $2x + 1$.

†The length of a single individual being taken as 200 μ .

left behind in the growth of the tip of the body, so that in the adult infusorian it lies halfway back to the middle of the animal (Fig. 3, ⁹). At the next fission it of course goes to the anterior product, lying at or behind its middle. By the greater growth of the anterior end it is further displaced backward, so as to lie clearly behind the middle. At the next fission it must then go to the posterior product, and be near its anterior end. Now it is again displaced slowly backward, the same processes being repeated. Thus the process is normally one of steady movement backward, interrupted by fissions which at intervals leave the spine near the anterior end of the posterior individual. A diagram showing this normal course of events is given in Fig. 7.

Sometimes through irregularities in growth, or other cause, the structure comes to be situated very near to or at one end (as in Fig. 4, ^{8,12,19-22}). Then the course of events becomes slightly different. If the structure is near the posterior end (Fig. 4, ⁸) the posterior tip grows back from it only a little, so that it still remains behind the middle of the body. At the next fission it therefore goes to the posterior individual (as it would in the "normal" course). Now the posterior end again grows back but a little, while the anterior tip grows much, so that the spine is still behind the middle. It therefore goes again to the posterior individual. It may thus require as many as three fissions to bring the structure to the middle, so that it passes again to the anterior individual, reestablishing the alternations (Fig. 4, ^{8 to 12}).

Is situated at or very near the anterior tip, the structure is carried forward in the growth processes; it may therefore remain for several generations in this region (Fig. 4, ^{19 to 22}), before it is displaced backward sufficiently to lie behind the middle. Possibly a structure might in the course of time attain a permanent position at the anterior tip. This seems indicated by the last three generations of *a*.

Thus on the whole the general tendency of the growth processes is to shift any surface structure from the ends toward the middle of the body, while the fissions again transfer it toward one end; with the further result of an alternation of position from the anterior to the posterior product of fission and back again.

In general then it must be realized that the parts of the body of the infusorian do not have a permanent definite relation to the form or structure. A portion of substance that is anterior in one generation is posterior or median in another. Thus definite

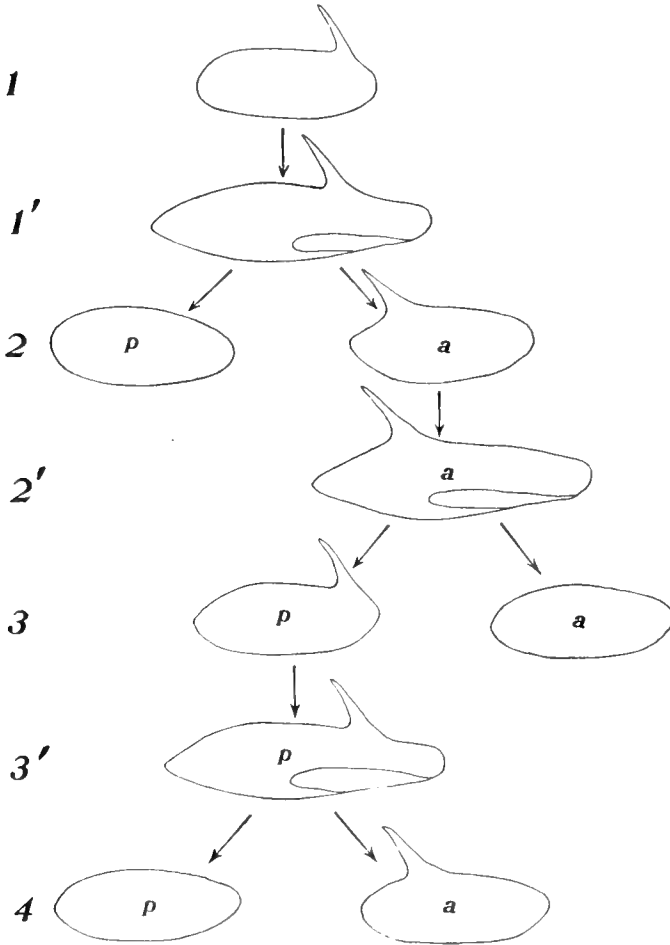


Fig. 7 Diagram showing the usual regular changes in form and alternations in position of the spine through four generations. The numbers at the left indicate the generations, a younger and an older stage being shown in each generation (save the fourth). *a* is the anterior product of fission, *p* the posterior one. In generations 1 and 3 the spine is on the anterior half of the posterior daughter cell; in generations 2 and 4 it is on the posterior half of the anterior daughter cell.

pieces of substance are not necessarily permanently differentiated to play certain parts. The organism is plastic, and is made over at fission. The normal reproduction involves the same working over and re-differentiation—"morphallaxis"—that occurs in regeneration.

3 Yet this making over is not complete. Oral and aboral surfaces retained their relative position throughout these twenty-two generations, the spine remaining always on the aboral surface. Furthermore, the entire history shows that a given structure may be bodily transmitted for many generations without becoming greatly changed. It may even, finally, acquire a more or less permanent position, remaining for at least several generations.

In the normal reproduction we find structures which behave in both of these ways—some being directly transmitted, others re-made. The two contractile vacuoles of *Paramecium* pass bodily, one to each of the progeny—though each individual forms likewise one new one. The mouth and pharynx are said to pass to the anterior product of fission, the posterior product forming new ones. The oral groove, the blunt anterior and the pointed posterior end, these are examples of structures that disappear in reproduction and are made anew. The cilia and setæ of the *Hypotricha* are not transmitted, but produced anew in the new individuals. Fission is on the whole mainly a process of reorganization and new production, rather than of transmission.

3 *Fate of Other New Structures in Reproduction*

The fate of many other new structural peculiarities was followed in various individual lines; after the detailed account we have given above, these can be set forth briefly.

a Spines, Points or Appendages

In many cases studied the history of points or appendages on the body differed from what we have described above for the line *a*.

1 This is the case with the small point on a^{11} , already mentioned. (Fig. 4, ³). As will be recalled, there resulted from the division of a^1 two individuals bearing spines or points; we have followed

the history of the large spine of $a^{1.2}$ and its descendants. We will now follow briefly that of the short posterior spine of $a^{1.1}$ (Fig. 8, ¹).

The next division (night of May 7) was of course at about the middle of the body, so that the anterior product $a^{1.1.1}$ was a normal individual without a spine. The posterior product $a^{1.1.2}$ had the spine in about the same position as in the previous generation, though it shifted during growth a little farther forward (Fig. 8, ²).

At the next (fourth) division the point passed to the posterior product ($a^{1.1.2.2}$) and remained in nearly the same position as before (Fig. 8, ³). It had become smaller, so that it was now a mere lump, hardly noticeable.

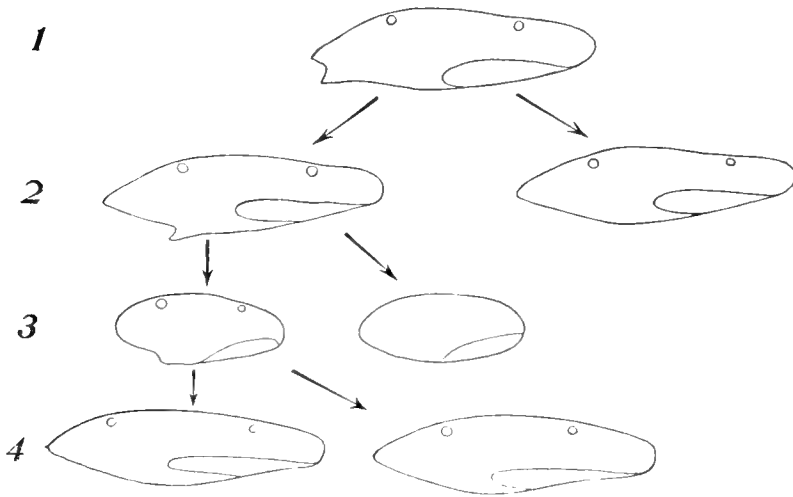


Fig. 8 Diagram of the history of the small tooth in the race *a*. See text.

At the next fission (fifth) the point or lump quite disappeared, being in some way reduced during the growth processes accompanying division. Both individuals resulting from fission were of the normal form (Fig. 8, ⁴).

Thus this small posterior point persisted through but three generations, and in each generation it was found in but one individual. A process of regulation of form took place slowly, accompanying the changes involved in fission, till finally the new structure had disappeared.

2 In a line or race which I called *am*, the course of events was as follows: The ancestor *am* was a short individual, seeming to lack almost completely the posterior half of the body. In the first two fissions the anterior product was in each case a normal individual, while the posterior product was more or less abnormal, with a blunt irregular posterior end. In the fourth genera-

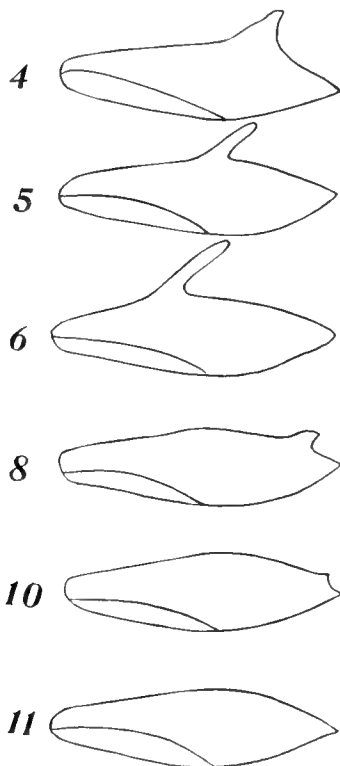


Fig. 9 A number of generations in the history of the race *am*, showing the shifting, transformations, and gradual disappearance of the spine. The numbers at the left indicate the generations figured. The spine first appeared in generation 4 and disappeared in generation 11.

tion there were two abnormal individuals, one of which bore a short spine projecting from its aboral surface, at about the middle of the posterior half of the body (Fig. 9, *).

In the fifth generation the anterior individual was normal, while the posterior one bore the spine a little farther forward than in

the previous generation. The spine itself was a little longer (Fig. 9, ⁵).

In the sixth generation it still further increased in length at the time of division, and went again to the posterior individual (Fig. 9, ⁶).

In the next two divisions the tooth went in each case to the posterior product, and continued to grow smaller. It remained near the posterior end, and in the tenth generation ($am^{2.2.1.2.2.1.2.2.2}$) it was hardly noticeable (Fig. 9, ¹⁰). During the next division it

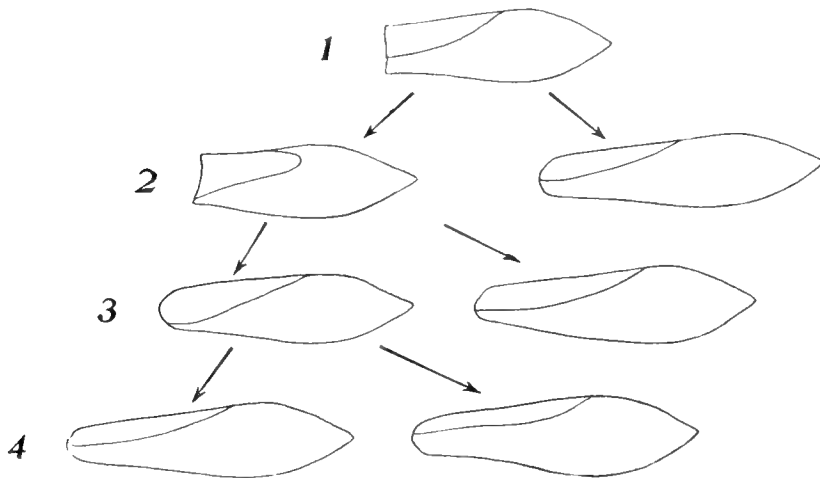


Fig. 10 History of a race derived from an individual with a truncate anterior end. The numbers indicate the generations figured. The truncate end is barely visible in generation 3, but had quite disappeared in generation 4.

disappeared completely, both products being typical individuals (Fig. 9, ¹¹).

Thus this spine persisted through seven generations, first increasing in size, then decreasing, till it disappeared.

b Anterior End Truncate

In three cases I followed the history of the progeny of individuals having the anterior end short and sharply truncate, as if cut off by a knife (Fig. 10).

In each case the truncation of the anterior end persisted for a few generations (two to five), being transmitted of course to but one individual in each generation. At each fission, as a rule, the peculiarity of the anterior end of this individual became less marked, till it became invisible. There is thus a marked tendency at the time of division to regulate the body form, bringing it back to the normal condition.

c Posterior Part of the Body Truncate or Lacking

Many individuals were found in which the posterior half of the body seemed almost lacking. The body ended bluntly just

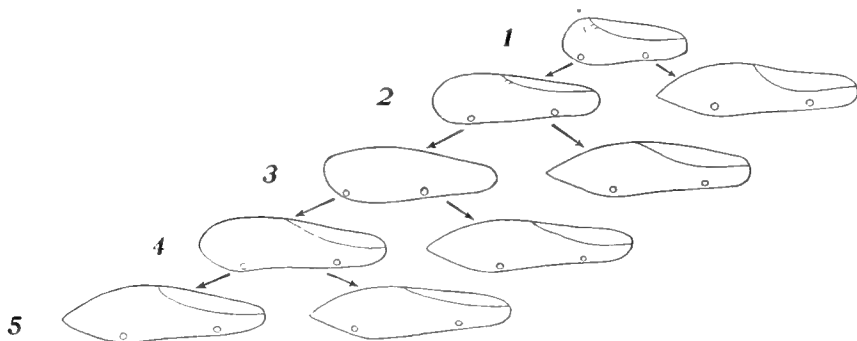


Fig. 11 History of a race derived from an individual in which the posterior part of the body was extremely short and rounded. The posterior end is to the left. The peculiarity was transmitted to one individual in each generation, becoming less and less marked, till in generation 5 it has disappeared.

behind the mouth. The animals were about half the normal size, and presented much the appearance that would result if they had been cut in two transversely just behind the mouth (Fig. 11).

I followed the history of ten cases of this sort. In all cases the bluntness of the posterior end is transmitted, usually in weakened form, to the posterior individual resulting from division, while the anterior individual is quite normal in form. This continues as a rule for three or four generations, the posterior end approaching after each division more nearly the normal form, till finally regulation is complete, and all the progeny have the usual shape. A typical case is shown in Fig. 11.

In one case the sharply truncate form of the posterior end was transmitted almost unchanged to the posterior progeny of the first divisions, though the posterior half of the progeny was much longer than in the parent. But in three more generations the posterior individual, like all the others, had reached the normal form.

d Anterior End with a Projecting Angle

In a certain culture there occurred a number of individuals in which the angle at the right of the anterior end was in a marked degree longer than others. These *Paramecia* ran over the bottom with the oblique surface of the anterior tip against the solid, suggesting that the projecting angle was due to this action. The angle disappeared in the changes connected with fission and did not reappear in the progeny.

e Crookedness or General Irregularity of Form

A considerable number of cases were studied in which the body of the progenitor was crooked, or was otherwise irregular in varied ways.

Such irregularities do not pass as such to the progeny. They usually cause modifications in some or all of the progeny for several generations, but these modifications are not repetitions of the parent forms. They result from abnormalities in fission due to the irregular form of the parent. Four categories of cases may be distinguished: (1) Those in which the irregularity of the ancestor induces in certain of the progeny various peculiarities that continue indefinitely; (2) those in which complete regulation finally occurs, all the individuals returning, after a number of generations to the normal form; (3) cases in which the result is to cause, in some or all of the progeny, still greater irregularities, resulting finally in monstrosities which cannot perform the vital functions properly, and therefore die; (4) cases in which the irregular individuals do not reproduce at all; they persist for a time, and finally die. Typical cases of each of these categories may be described.

1 The individual *a*, whose history has already been followed (pp. 589-604), is an example of the first category. Here the crooked-

ness of the parent (Fig. 4, ¹) caused a spine to appear on one of the progeny; this persisted on a single member of each generation, as long as it was followed (22 generations). The other progeny were normal.

2 The individual *al* was bent a little in front of the middle so as to form nearly a right angle (Fig. 12, ¹). At the first division the posterior product was of the normal form, while the anterior product was somewhat irregular (Fig. 12, ²) but less so than the parent. When this divided, the two individuals resulting were both of the normal form. Regulation occurs during the process of fission.

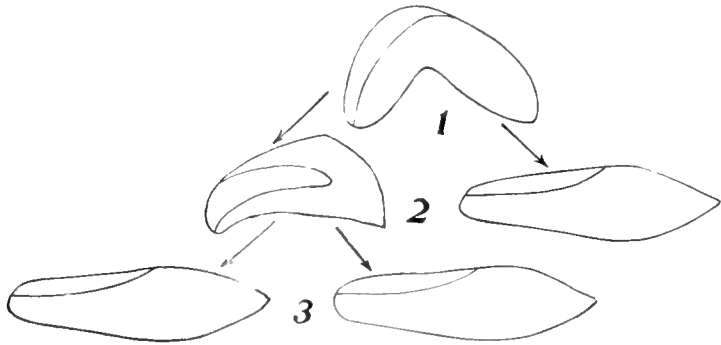


Fig. 12 History of a race derived from a crooked specimen. The crookedness had disappeared in the third generation.

The individual *ab* had the posterior end crooked (Fig. 13). When this animal was placed in the culture fluid, it became plumper, and the abnormality of form was less marked (¹). When it divided the anterior product was of the normal form, while the posterior product had the posterior point slightly displaced toward the aboral side, but was otherwise normal (Fig. 13, ²). When it again divided, its progeny were both normal in form.

The case of *aj* belongs partly in the second category, partly in the third. The body of the parent *aj* was small and irregular, with a broad anterior end, which bore on one angle a projecting point (Fig. 14, ¹).

When this was placed in the culture fluid it did not divide for three days. The body increased in size and especially in thick-

ness, and the projecting angle became more marked (Fig. 14, ¹). On the third day it divided; the posterior product was normal in shape, though smaller than usual, while the anterior product was extremely irregular, having the form shown in Fig. 14, ².

In the next twenty-four hours this irregular structure underwent a partial division, increasing its size and irregularity of form (Fig. 14, ³). The structure thus produced was double, since it had two mouths (*m*), both of which took food; and there were two independent protoplasmic circuits for the digestion of food.

During the next twenty-four hours this structure divided into two very unequal parts. One product was a short, somewhat

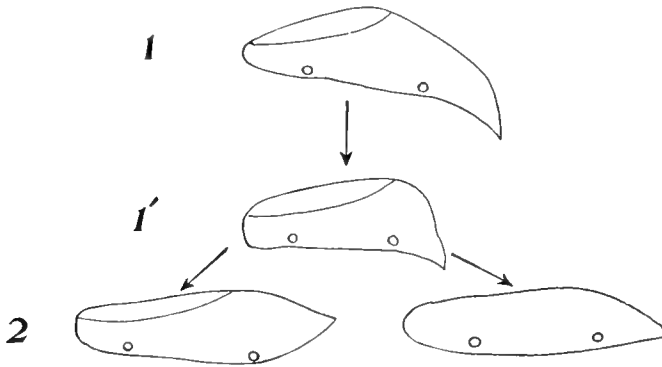


Fig. 13 History of a race derived from an individual with a crooked posterior tip. The irregularity had nearly disappeared in the second generation; in the third (not shown) it was quite gone.

irregular individual. The larger product was still very irregular; it represents three united individuals (Fig. 14, ⁴).

The smaller product divided again, producing progeny that were normal in form, though small in size.

The larger product, composed of three incompletely separated individuals, did not divide again; after two days it disintegrated.

3 The individual *aq* represents mainly the third category, in which the irregularity of form is increased in reproduction, till death occurs. This specimen was curved as shown in Fig. 15, *a*. At its first division the products did not completely separate, but formed the structure shown in Fig. 15, *b*. At the next division the right half divided in such a way as to produce one nearly nor-

mal free individual, while the other product remained attached to the left half. The latter underwent a partial, irregular division. Thus the result is to produce an irregular structure consisting of three fused individuals (Fig. 15, *c*).

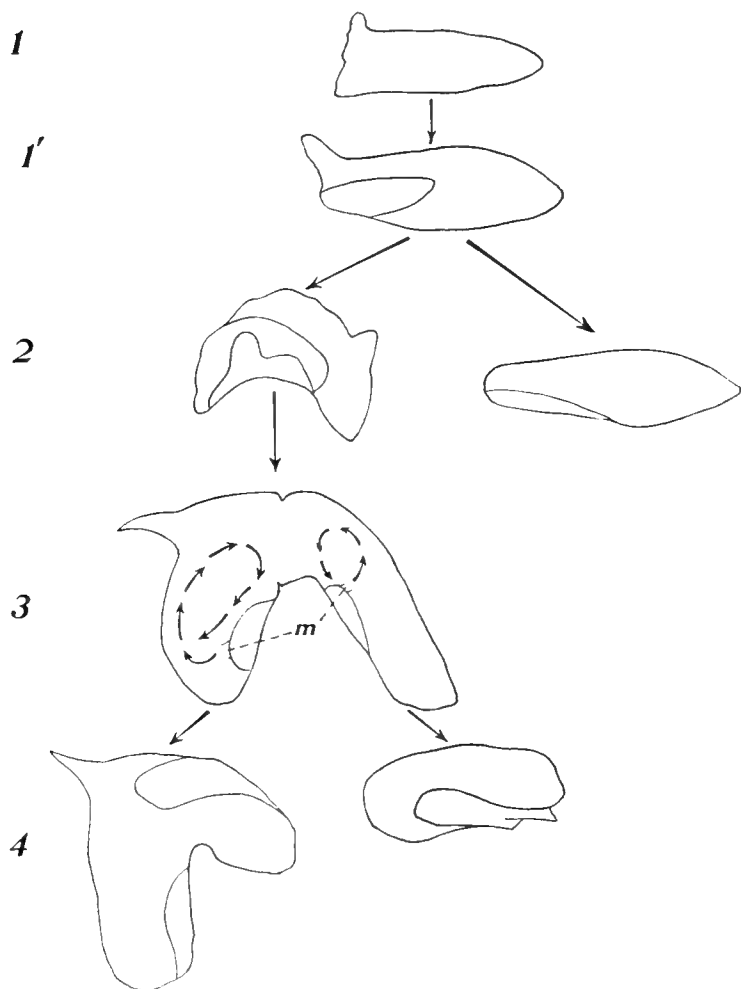


Fig. 14 Diagram of the history of the race derived from the irregular individual *aj*. In the third and fourth generations double and triple monsters appeared, with several mouths (*m*) and multiple proto-plasmic circuits. Two such circuits are shown by arrows at 3.

This structure underwent other partial fissions, giving the irregular monster shown in Fig. 15, *d*. This lived for about four days, then disintegrated.

4 Instances of the fourth category, in which no divisions occurred, are given by *aq*¹ (Fig. 16, *a*) and *am*^{2.2.2} (Fig. 16, *b*).

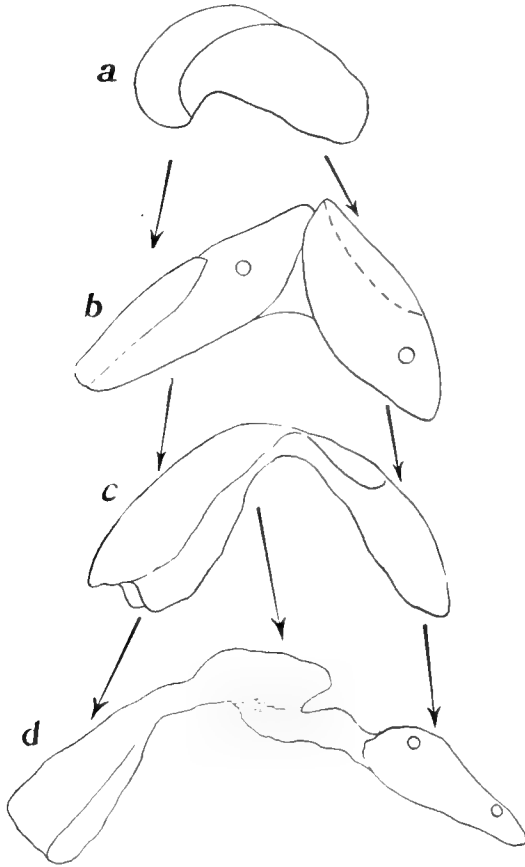


Fig. 15 History of the race derived from the irregular individual *aq*. See text.

These both lived for five days without dividing or taking food; both then disintegrated.

The mass *ar* was the result of partial fission, so that it included several partial individuals. As successive partial fissions occurred

it took various forms, of which the three given in Fig. 16, *c*, *d*, *e* are types. This structure took food by five or six mouths, and had a number of partly independent systems of circulation. It reached a length of 450μ , with a breadth of 150μ . The normal *Paramecia* in the same culture in which it occurred showed dimensions of about $150\mu \times 60\mu$. This structure had therefore the bulk of about twenty normal individuals.

This was kept for ten days, but finally it disintegrated.

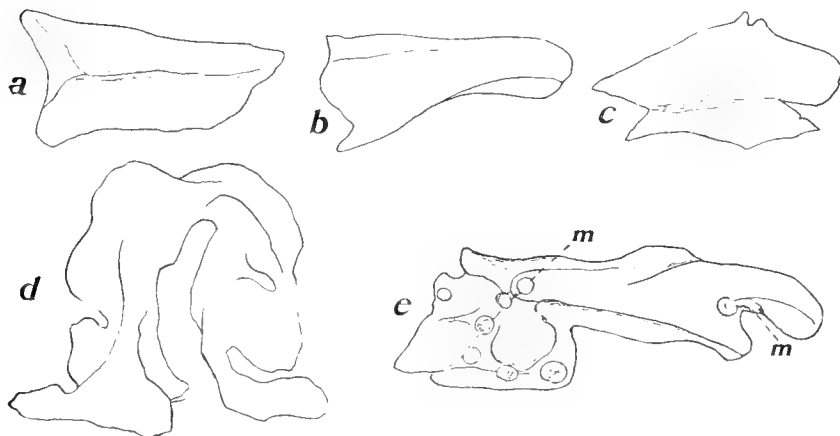


Fig. 16 Irregular individuals which do not divide farther. *a* and *b* are separate individuals that finally died. *c*, *d* and *e* are stages taken at intervals of several days in the complex mass *ar*. *m*, mouths.

f Behavior of Mutilations in Reproduction

Paramecium differs from many of the infusoria in the fact that it does not stand mutilation well. The internal contents seem very fluid, so that they flow out as soon as the ectosarc is cut; the animal at once disintegrates. It is therefore difficult to study the regulation of injuries in this animal, either during the active life, or at reproduction.

However, from a large number of experiments, certain results were reached that show how mutilations behave, both in ordinary regulation and in reproduction.

1 *Mutilations in adults.* Whenever the ectosarc is punctured, the internal contents flow out and the animal dies. But in a few cases mutilations were produced without puncturing the ectosarc.

Thus, a fine glass rod was drawn across an individual near its middle; leaving a deep constriction, while the two halves of the body were swollen (Fig. 17, *a*). This constriction persisted for some hours, becoming gradually less marked. The next day the animal was perfectly normal.

In another similar experiment, blister-like swellings were produced, and the anterior portion of the body became totally irregular (Fig. 17, *b*). But within 24 hours the normal form was completely restored.

Thus it is clear that the adult *Paramecium* has the same power of regulating form that is so well known in *Stentor* and other infu-

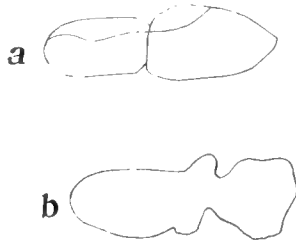


Fig. 17 Mutilations produced by drawing the tip of a glass rod across the adult animal. See text.

soria. But this can come into play only when the injury has not been of a nature to puncture the ectosarc and so to cause disintegration.

Many attempts were made to remove only a part of the internal fluid (endosarc), without causing death. The ectosarc was pierced with the tip of an excessively fine capillary glass rod.* But in all cases where any of the endosarc flowed out, the remainder followed, and the animal died.

2 *Mutilations in dividing specimens.* It was thought possible that specimens undergoing fission might show a different physical state of the protoplasm, such as to permit mutilations without immediate disintegration. To a limited extent this was

*These can easily be made so fine that the tip is apparently not larger than a cilium of *Paramecium*.

found by experiment to be true. When a specimen undergoing fission is pierced with the tip of the glass rod or otherwise mutilated, it does not go to pieces so rapidly as the adult, though in most cases it finally disintegrates. But in a few instances specimens thus treated survived.

Thus, while the *Paramecium ma* was undergoing fission, its anterior half ma^1 was pierced with the rod, allowing a part of the internal contents to escape. This half became distorted (Fig. 18, *a*) while the other half became swollen. The latter resumed later its normal form, and fission continued. The injured half *a* retained its distorted form (Fig. 18, *b*). During growth the form became somewhat nearer normal (Fig. 18, *c*), but complete regulation did not take place in this generation.

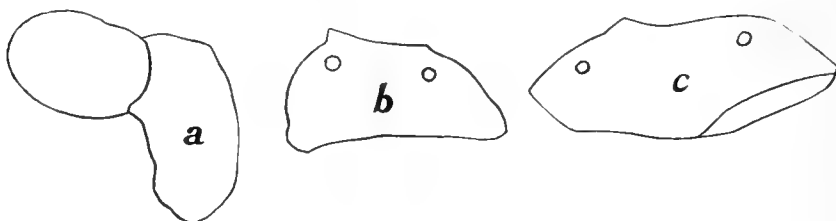


Fig. 18 History of the specimen *ma*, mutilated during fission. See text.

During the night the irregular individual divided. The anterior product was quite normal in shape; the posterior one still showed a slight irregularity of form at the posterior end. At the next fission this disappeared and both products were normal.

Thus the effects of the mutilation persisted in some of the individuals for three generations, then disappeared.

In a number of other cases young or dividing specimens were marked with deep furrows by pressing them with the rod. These marks lasted some hours, but disappeared before the next fission occurred.

In the dividing specimen *mb* the posterior part mb^2 was pierced with the glass rod, so that a part of its contents escaped, while by contraction most of the remainder of its contents were forced into the anterior half mb^1 (see Fig. 19, *b*). Thus the

pierced part became very small; later it increased in size and became irregular (Fig. 19, *c*). The fission was never completed, this irregular part remaining attached to the posterior end of the normal individual *mb*¹.

The normal part *mb*¹ divided twice, budding off, as it were, two normal individuals at its anterior end; its posterior part remained with the irregular mass attached, as in Fig. 19, *d*.

At the next division the two components remained connected, with the irregular mass attached to the posterior end (Fig. 19, *e*).

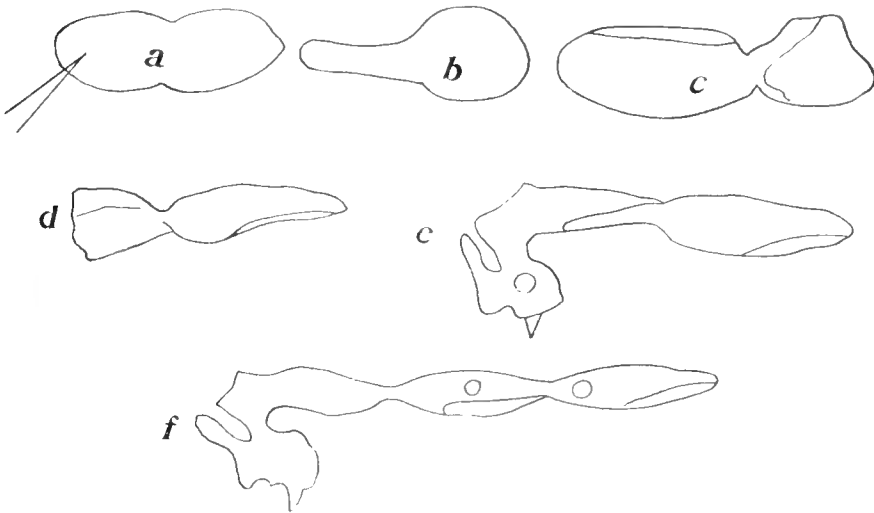


Fig. 19 Effect of mutilation during fission in the specimen *mb*. See text.

The irregular mass had itself made some attempts at fission, with the result that it became still more irregular.

There was no further change for three days; then another partial fission produced the results shown in Fig. 19, *f*.

During the next day the entire structure disintegrated. In this case the effects of the mutilation lasted for several generations, finally causing death.

All together, it is clear that while mutilations may be passed on bodily to certain of the products of division for a number of generations, there is no tendency for them to be inherited by all the

progeny; no tendency for the mutilation to be duplicated in new individuals. There is no tendency to produce a race of mutilated individuals, any more than there is in Metazoa. Regulation takes place at the time of fission, so that after several fissions the normal condition is restored.

4 *Acquired Characters That Tend to be Inherited*

g *Acquirement and Inheritance of a Tendency for the Adults to Remain United in Chains*

The acquired characteristics thus far described have shown no tendency to be inherited in such a way as to produce a race bearing the new character in question. We now come to a case in which such a tendency actually showed itself. The difference between this case and the others is instructive, suggesting what must be the essential nature of an acquired character that may be inherited.

The characteristic in question is a tendency for the adult individuals to remain united in chains. This tendency appeared in the line *a*, which we have already described in connection with the transmission of a long spine (pp. 589-604); the beginnings of the characteristic now under consideration have been set forth in that description. In the process of growth the broad base of the long spine (Fig. 4, ⁷) became drawn out, till in the individual *a*^{1.2.1.2-1.2.1.2} it formed a ridge running along the aboral surface nearly the entire length of the body (Fig. 4, ⁹). At the next fission it was found that the fission plane did not pass so readily through this ridge as through the remainder of the body, so that the two resulting individuals did not separate, but remained connected by a bridge passing from the aboral surface of one to that of the other (Fig. 4, ¹⁰).

The continued union of the two individuals after fission reappeared in succeeding generations, both in the individuals formed from the region anterior to the spine (as in Fig. 4, ¹⁰), and in those formed from the region posterior to the spine (Fig. 4, ^{17, 19}). In the eighteenth and twenty-first generations three individuals formed a chain (Fig. 20, *a*). In succeeding generations many such connected individuals and chains were formed. In the fif-

teenth generation I began to save all the progeny of *a*; up to this time only the specimen bearing the spine had been kept alive. In the large number of progeny thus obtained many variants were to be observed in the matter of interconnection. Many individuals were free and separate. Pairs of united individuals were very common. Chains of three to eight or more (Fig. 20) were not uncommon. These longer chains were likely to break apart in the course of time, as a result of their bending and twisting movements.

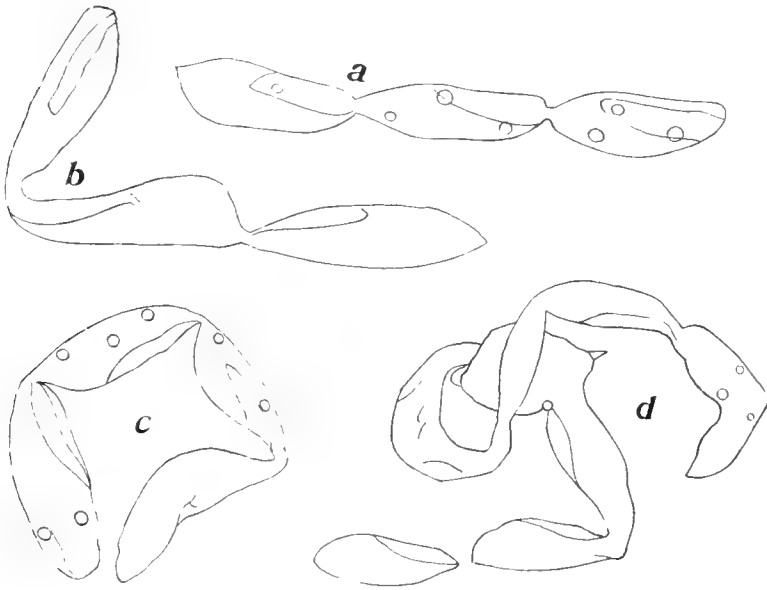


Fig. 20 Chains of individuals formed in the history of the race *a*, as a result of incomplete fission.

There was much variation in the extent and strength of the union. Sometimes there resulted from the division of united individuals specimens that were quite free. The division of free specimens often produced united pairs. In some cases the connecting band was very thick and strong, so as to hold the two specimens inflexibly in various positions (Fig. 20, *b*). In other cases the fission was so incomplete that mere partly double specimens resulted (parts of *d*, Fig. 20). Finally, the irregularities

of fission at times went so far as to produce mere monstrosities (parts of *d*, Fig. 20). Such monstrosities were rare, while individuals neatly united in pairs or in chains were very common.

The first occurrence of such unions (Fig. 4, ¹⁰) was on May 10. Cultures were kept in watch glasses from that time till July 1 (probably about fifty generations); at that date the unions were still abundant. In fifty generations the original individual which underwent the modification causing the union would have produced progeny to a number running far up into the billions.

Effects of Artificial Selection

On June 22 I began experiments to determine the effect of selection on this peculiarity. Would it be possible by selection to produce on the one hand a series showing little or no tendency to remain united, on the other hand a series in which most or all the individuals remain in united pairs?

Two selected cultures were started in watch glasses. The first contained twenty individuals united two by two in ten pairs. The second contained twenty free individuals (descended from the same ancestors as the united pairs).

Forty-eight hours later (June 24), both sets had multiplied to about 100 specimens. In the first set (ancestors united) there were ten united pairs. In the second set (free ancestors) there were two united pairs.

From the first set I removed all the free individuals, leaving only the ten united pairs. From the second set the two united pairs were removed, leaving all free.

The further history was as follows:

Culture from free ancestors. On June 25 this had multiplied to 200-400; among these were three or four united pairs. I removed the latter and retained only 100 of the free individuals.

On June 26 these had multiplied two to four times but contained no united specimens. This culture was kept for a week or so longer, but developed no more united pairs. Thus, selection had quite removed from this set the tendency to remain united.

Culture from united ancestors. After the second isolation of ten united pairs (June 24), the number multiplied to about 50 in 24

hours; among these there were eight groups of united individuals—some of two, some of several, specimens united in chains. The eight groups were again isolated (June 25).

Effects of Natural Selection

These eight groups showed many imperfect individuals, and the groups were at a great disadvantage as compared with the free individuals. This was because they are not able to swim about actively, but must lie at the bottom and move about only irregularly. As a result they get comparatively little food, and are not able to avoid regions where the conditions are harmful. The bacteria multiplied much more rapidly than in the free culture, containing many individuals—the latter keeping down the number of bacteria by feeding on them.

In consequence of these bad conditions, the united groups began to die. Some multiplied farther, all the individuals remaining united. But forty-eight hours after the isolation of the second lot of eight groups, all were dead.

Thus it is easy to produce by selection a culture containing only free individuals and multiplying in the usual way. Artificial selection will likewise produce a culture of united specimens, multiplying mainly by incomplete fission. But at the same time natural selection acts; these groups die, owing to their inefficiency in getting food, keeping down the bacteria, avoiding harm, and in the performance of the general bodily functions.

This extinction by natural selection of the series multiplying by incomplete fission was shown in another way. A considerable number of the progeny of *a*, with both separated individuals and united groups, was allowed to accumulate in a shallow watch glass. Here the united groups flourished fairly well, because the vessel was so shallow that they received plenty of oxygen and of food while lying on the bottom, while the undue multiplication of the bacteria was prevented by the numerous free individuals. Now the culture was transferred to a large vessel, three inches deep. Here the culture multiplied enormously, but all the groups of united specimens quickly disappeared. They sank to the bottom

of the vessel, where the conditions were not such as to keep them alive, while the free individuals remained at the top and multiplied. Thus by continued natural selection all specimens multiplying by incomplete fission were removed, and in a few days the deep culture contained only normal, free individuals. In shallow cultures, on the other hand, the united groups persisted for about two months, as we have seen.

In this case then we have a new characteristic, of known origin, that is inherited by many individuals for many generations, and is finally extinguished only by the action of natural selection. The many other new characteristics that we have described were not inherited (save as they were handed on directly to a single specimen). In the one case the new feature becomes a race characteristic; in all the others it fails to do so.

WHAT MUST BE THE NATURE OF A NEW CHARACTER, THAT IT
MAY BE INHERITED ?

What is the peculiarity of the characteristic that was thus multiplied and inherited, and what light does it throw on the question as to what must be the nature of an acquired characteristic in order that it may be inherited ?

The characteristic thus inherited was *a modification of the protoplasm of the cell, of such a character as to cause it to behave differently in reproduction*. The other characteristics, not inherited, were not such modifications of the protoplasm as to cause it to behave differently in reproduction.

Consideration of the facts of normal reproduction in the Protozoa, and of heredity in general, indicates that this difference is an essential one. *In order that it may be inherited* (by more than one of the progeny), *a characteristic must be the result of such a modification of the mother cell as will cause it to behave in a certain way at reproduction*. It makes no difference whether the mother cell in question is a germ cell, in a Metazoan, or a differentiated Protozoan.

Thus we know that in the inheritance of the setæ of the Hypotricha, for example (Fig. 21), these are not simply handed over in

finished form, like the spine of *a* (Fig. 4), but are *produced anew* on each product of fission. The old setæ and cilia degenerate and disappear as fission sets in. In the daughter individuals the new setæ appear in a small group with a totally different arrangement from that seen in the adult parent (Fig. 21, *x*) and the final arrangement is reached by complicated processes of differentiation and distribution. Thus the presence of setæ in the posterity could have been brought about in the beginning only by such modifications of the protoplasm of the mother cell as would cause it at fission to *produce setæ*. Any change in the structure, number, or

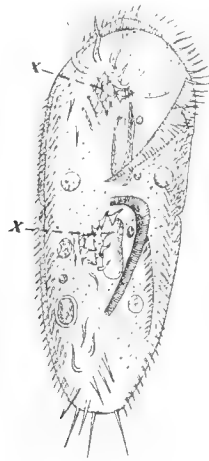


Fig. 21 Dividing *Styloynchia*, from Bütschli, showing at *x* the appearance of the new setæ in a close group

arrangement of the setæ could result only from such a modification of the mother cell as would alter in a definite way the processes occurring at reproduction. The thing transmitted from the parent cell to the young progeny is, not the setæ themselves, but the change in the protoplasm causing the production of setæ in a definite way.

To return to a specific problem—How then could such a localized appendage as the spine of *a* (Fig. 4) become an inherited characteristic? *Only through such a modification of the protoplasm*

of the parent cell as would cause at fission the production of such an appendage on each of the progeny.

At first thought it appears difficult to conceive how this could occur. This will be made easier, perhaps, by a consideration of the origin of certain characteristics in the race *a* (Fig. 4, etc.).

Examples of Modifications from which New Inherited Characters Might Result

Let us take first the origin of the spine whose history is traced in Fig. 4. The original ancestor of the race *a* was without spines. But it was so deformed and modified that at the time of fission two short teeth were produced during the processes of division (Fig. 3, ²). At the next fission one of these short teeth formed as it were a region of weakness, where a long spine was pushed out, as an accompaniment of the processes of fission (Fig. 3, ^{4, 5}). Such a region of weakness might well exist without a visible tooth to show its position; then at fission a spine would be produced in this spot. It is evident that active physical and chemical processes are in progress at the time of fission; these may easily result, under the influence of a local modification of the parent cell, in the pushing out of a spine or other structure of characteristic form.

How such a new structure might appear in each of the progeny of each generation is illustrated in a simple way by certain other phenomena seen in the race *a*. As we have already set forth, the progeny of *a* showed after a certain period a tendency to remain united in chains. At the same time there appeared among the free progeny of *a* a considerable number of individuals which bore at one or both ends a spike-like point (Fig. 22). This character did not become general, but so many cases appeared that one might say that there was an inherited tendency toward this. Observation of the process of fission showed that these points arose by the pulling out of the protoplasm while in the plastic condition at the time of fission; the two young animals were connected, at a certain stage, by a bridge of this plastic protoplasm. By their movements they drew this out to a long strand, which finally broke at the middle, leaving a point at the ends of the two animals.

When this happened at successive fissions, the animal bore such a point at each end.

It is evident that these points are due to the same cause that produced the inherited tendency to remain united in chains (as in Fig. 20). They result from the ridge of new material along the aboral side of the animal, shown in Fig. 4, ⁹. Now, it is easily conceivable that this new material might be of such texture and thickness that it would always be drawn out at fission in such a way as to produce points of a definite form and size. These would then appear regularly after fission; a race of *Paramecia* with this as



Fig. 22 Examples from the race *a*, of individuals having a point at the posterior end, due to the drawing out of the connecting band at the time of fission.

a new characteristic would have been produced. The spine would be hereditary, because produced anew in each generation, just as are the setæ of the *Hypotricta*, or the organs of the Metazoa.*

SUMMARY AND GENERAL DISCUSSION

The following general statements of the laws and principles bearing on heredity† that result from our investigation are made with direct reference to the Protozoa, and will best be grasped by keeping in mind concrete cases, such for example as those shown in Fig. 4, Fig. 20 and Fig. 22.

*It is of course possible that the origin of new permanently inherited characters is not normally through mere modifications of the external parts of the cell, such as we see in our illustrative cases. Possibly there must be originally some modification of more recondite parts—nucleus, chromosomes, or the like—and that these then secondarily act upon and change the outer parts. This would add farther complication, but would not change the essential point, which is, that in order that a characteristic may be inherited, it must be due to some modification that causes a change in the processes of reproduction.

†For a summary of results on other matters than heredity (on the changes during fission and growth, etc.), see pp. 599–604.

1 The "inheritance of acquired characters" meets the same difficulty in the Protozoa as in Metazoa. In both Protozoa and Metazoa most characteristics acquired during the lifetime of the individual are not inherited, and such inheritance does not occur more readily in the one group than in the other.

2 The difficulty with the "inheritance of acquired characters" lies, not in the separation of soma and germ, but in the process of cell division. If a cell bears a structure at one end, there is no simple and direct reason why, when it divides, *both* the cells produced should bear the structure, and observation shows that *they do not*, in the case of new structures. There is no evident way in which a structure of this sort can overleap the barrier of cell division and appear on the other side.*

If we insist on making a comparison between the condition in the Protozoa and the separation of soma and germ in the Metazoa, the following is the state of the case. If any Protozoan cell (as in Fig. 7) is to be divided at the next fission into two parts *a* and *p*, then, so far as inheritance of new structures is concerned, *a* stands to *p* as soma to germ, and reciprocally, *p* stands to *a* as soma to germ. In other words, there is no evident transmission, and no evident mechanism for transmission, of a new structure from *a* to *p* or the reverse, just as there is no evident mechanism for transmitting a structure from soma to germ.

3 In order that a character may be inherited (by more than one of the progeny, so as to produce a race), it must be *produced anew* in each generation. This is what happens in the normal reproduction of both Protozoa and Metazoa.

4 In order that a new (or "acquired") character may be inherited, it must be the result of such a modification of the parent cell as will cause a change in the processes of reproduction; and specifically, precisely such a change in these processes as will produce the character in question. This is equally true of Protozoa and Metazoa.

5 Most characteristics acquired during the life-time of the

* This will be most readily grasped by looking at the figure of a typical case, such as Fig. 4, 3. Why, when this animal divides transversely, should there be a spine upon the posterior (left) half, as well as upon the anterior (right)? As a matter of fact, there is *not*.

individual are not the result of such modifications of the parent cell as will cause a change in the process of reproduction such as to produce anew these characteristics; hence they are not inherited. This is true in both Protozoa and Metazoa.

6 Thus the problem of how new inherited characters arise is the same in Protozoa as in Metazoa. We may therefore work on the general problem as readily in the one group as in the other, and there is no reason why the principles reached in one group should not apply equally to the other. Thus a new line of attack on the problem is opened; in view of the rapid multiplication of the Protozoa and the ready accessibility of their reproductive cells both to environmental influences and to observation, this gives some marked advantages.

7 The search for the origin of new inherited characters (in both Protozoa and Metazoa) resolves itself experimentally into a search for agencies and processes which will permanently modify the cell in such a way as to cause it to act differently in reproduction.

8 When a given structural characteristic arises during the reproductive processes so as to appear in a given generation, that is not because the same structure was present in a preceding generation. Often indeed it was not present before; its origin is due to some change in the constitution (chemical or structural?) of the preceding reproductive cell. Thus, the production of a spine such as we see in Fig. 4 is evidently due to a spot of weakness at a certain point in the cell body, causing a protrusion during fission. Such a structure might result from the localized presence somewhere in the cell body of a certain chemical compound, which would react at a certain stage with some other substance, thus producing a spot of weakness, where a spine would be protruded. So, the appearance of the new anterior setæ in the posterior product of division in the *Hypotricha* (Fig. 21) is evidently due in some way to the constitution of the cell.

9 Thus, then, the cause of the appearance of a certain structure in a certain generation is *some other peculiarity* of the cell producing it; some chemical peculiarity, for example. We may generalize this by saying that the appearance in the progeny of a certain structure *b* is due to the existence in the mother cell of a quite different condition *a*.

10 It follows from what has been set forth in the paragraphs preceding, that in the production of a new inherited character the original modification will be something quite different from the visible structural characteristic which later appears in consequence of it. The original modification will be some chemical or structural change in the reproductive cell or cells that are later to produce the structure in question. (By producing in *Paramecium* a localized change in the character of the protoplasm, a spine is later produced at that spot, etc.) The first appearance of the visible structure is *one generation after* the production of the modification to which it is due.

11 Not all modifications of the germ cells that result in the production of a new character in the *next* generation, will result in the repeated production of this character in succeeding generations. In most cases, the new structure appears *but once*, and is not inherited. In order that the new structure shall be inherited, the original modification to which it is due must be *transmitted* to the succeeding generation of germ cells. This is by no means a matter of course; in fact, it is something *not to be expected*, as a rule. The cell usually, by regulative processes, throws off after a time any modification which the environment has impressed upon it. Many examples of this are seen in the foregoing pages. Certain unusual conditions of the cell result in the production, at the next fission, of a spine. But during fission regulation occurs; the unusual condition disappears, and the spine is not again produced.

This is doubtless the fate of most modifications of the cell. We saw, however, one modification which persisted, producing its effect in succeeding generations (pp. 618-622). Of such a nature must be all modifications which produce new inherited characteristics. It is easy to so modify the cell that new characteristics shall appear in *one* succeeding generation; to so modify it that the new characteristic shall appear regularly in succeeding generations is a totally different matter.

We often hear it pointed out that *heredity is not transmission*, but new production; and this has been emphasized in the preceding pages. But it needs to be realized that while it is true that

the inherited structure visibly appearing is not transmitted, *something is transmitted*, namely, the condition of the protoplasm which causes the production of the visible inherited structure. If this determining condition were not transmitted, the visible structure could not be produced in each generation. It is this "something" transmitted that lies at the basis of the figurative expression "bearer of heredity," or the like.

12 What sort of modifications will remain permanently and be transmitted to the progeny? Evidently, only such modifications as are not removed by the regulatory processes of the cell. The modifications that are removed by regulation are precisely those which interfere in one way or another with the physiological processes of the organism, while modifications which arise in harmony with, or as a result of, the normal functioning of the cell are not removed by regulation. Thus only characteristics of the latter class—namely, *adaptive* characteristics—will be retained and transmitted. Furthermore, it appears clear that the successive modifications in the reproductive processes induced by these adaptive characteristics must likewise be in harmony with the normal functioning of the cell, else they would be removed by the known regulatory activities of the cell. Thus all stages in the modification, including the final one, must be in harmonious adjustment to the normal activities of the organism. It would appear therefore that only the new characteristics that are *adaptive* will be inherited. Anything not in harmony with the normal functioning of the cell will be removed by regulation.

13 Let us now examine the problem of the "inheritance of acquired characters." What processes would be required for the inheritance by the progeny of the same characteristic that has already been produced *directly* in the parent, by environmental action?

Keeping the Protozoa in mind, we have evidently two cases here:

a If the "acquired character" is some *general* chemical or structural change in the parent cell—something that affects the cell as a whole—then there appears to be no special difficulty in the way of a direct transmission of this to the progeny, provided

it is not thrown off by regulation. If new inherited characters of any sort are ever produced by environmental action, such direct transmission of an acquired internal modification must occur, as we have already seen (paragraph 11). In the Metazoa, it would evidently be only general changes in the *germ cells* that would be thus directly transmitted.

b The case of a new *localized* modification or of a definite new structure, such as a spine, which is directly produced by environmental action, is wholly different. As we have already seen (paragraphs 9, 10, 11), in order that a new localized structure *b* shall appear in each generation, a certain other condition *a* must be produced in the mother cells; this condition *a* must be transmitted from generation to generation, and must so modify the reproductive processes as to cause, at each fission, the production of the new structure *b*.

Now, if the new structure *b* was first produced *directly* in the parent by environmental action, and is then to be inherited, the processes required are the following. The existence of the structure *b* (a spine, for example), in the parent cell, must cause the production in that parent cell of precisely the "other" condition *a*, that is of such a nature as to so change the processes of reproduction that they will again produce identically the character *b* (the spine) which had first been produced by the environment. Or, what amounts to practically the same thing, the environment must coincidentally produce two heterogeneous effects: (1) it must directly produce the structure *b*; (2) it must produce some permanent change *a* in the constitution of the cell, such as will so modify the processes of reproduction that they in their turn will produce the same structure *b*.

Such coincidental production of a complex structure *b* in two quite heterogeneous ways would be most extraordinary, and we have as yet no glimmering of a mechanism by which the coincidence could be produced. Moreover, as we have seen, in most cases (in *all* precisely observed cases) it is *not* produced; we have little if any direct evidence that it ever occurs.

Yet if it *ever* occurred it would be of such importance that we must of course continue to be on the watch, in all experimental

work, for any evidence of it. The question, put as simply as possible, is as follows:

Is there ever any mechanism or property in virtue of which, when a structural modification occurs in one part of the body, this will modify another part of the body (not in the same way, but) in such a way that this other part will, at reproduction, start up processes tending to produce a similar structural modification?

14 The propositions thus far set forth have had direct reference to the Protozoa; but in the main they apply *a fortiori* to the Metazoa also. The difference between the two groups as to heredity is not one of principle, but of complexity. The extreme difference in complexity may be put as follows:

In the Protozoa, when a new inherited character is to appear in the adult, this requires a modification of the adult of the previous generation, of such a character as to change in a definite way only the next fission and processes immediately connected with it. This requirement is sufficiently complex when we come to ask how the numerous locomotor organs of the *Hypotricha*, in their varied typical patterns, have arisen and become hereditary. But it is not to be compared in complexity with what we have to set forth next.

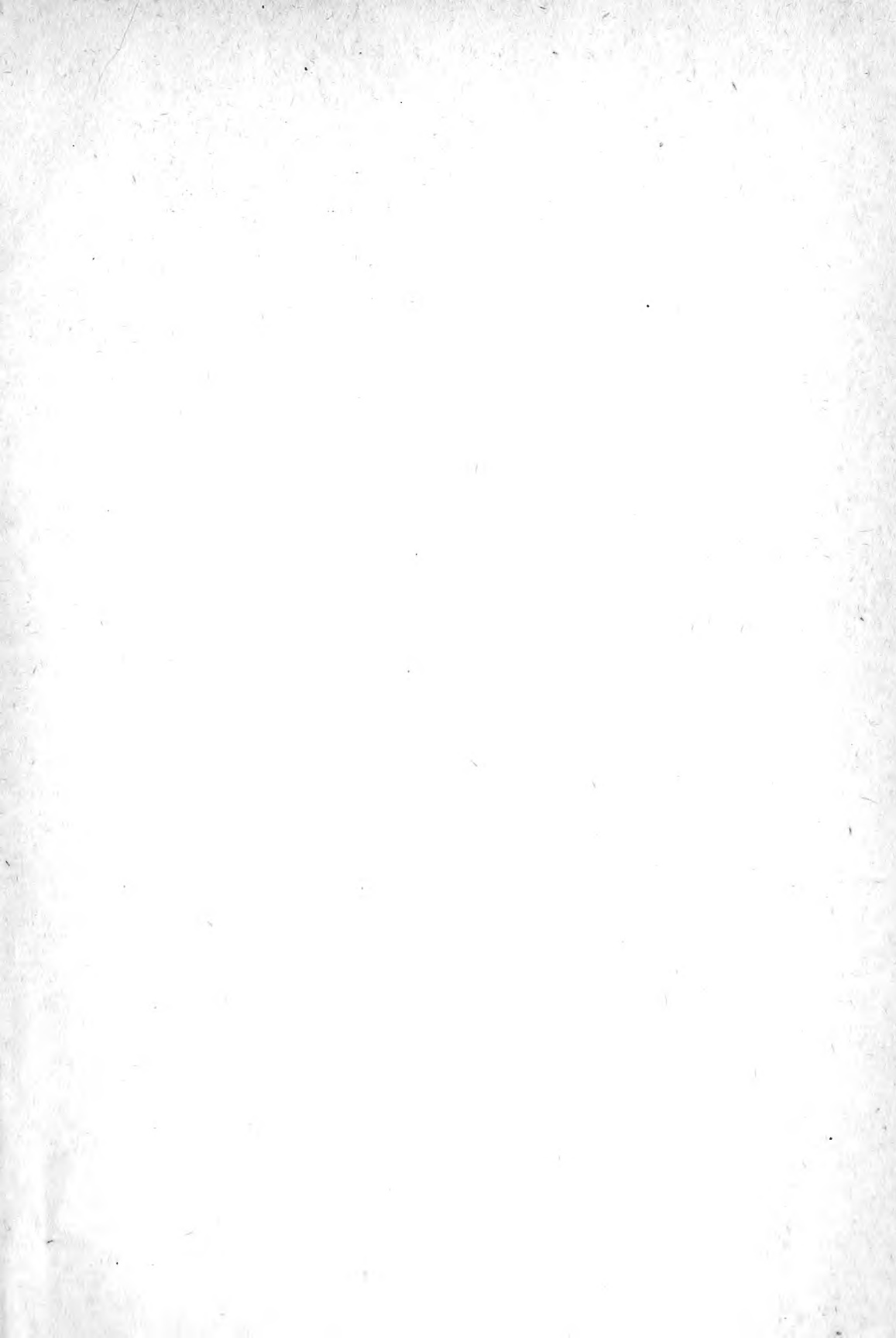
In the Metazoa the requirement for the appearance of a hereditary new structure in the adult is that the preceding germ cell shall be so modified that at the next fission the reproductive processes shall be changed, but the change shall not yet be of a character to produce the ultimate structures. In the next and the next, and in hundreds of succeeding fissions the processes must all be modified so as to keep in each cell the conditions for the final production of the ultimate new structure. These conditions will necessarily be different in the different cell generations, as differentiation occurs, and of course each of the intermediate conditions is something quite diverse from the final structure. At the end the new structure is produced, not by a modification in the reproductive processes of one cell, as in the Protozoa, nor by the *same* modifications in many cells, but by the diverse modifications of thousands and thousands of cells, all so modified as to coöperate in the production of the final structure. The mind refuses the useless attempt to conceive of such complexity of change.

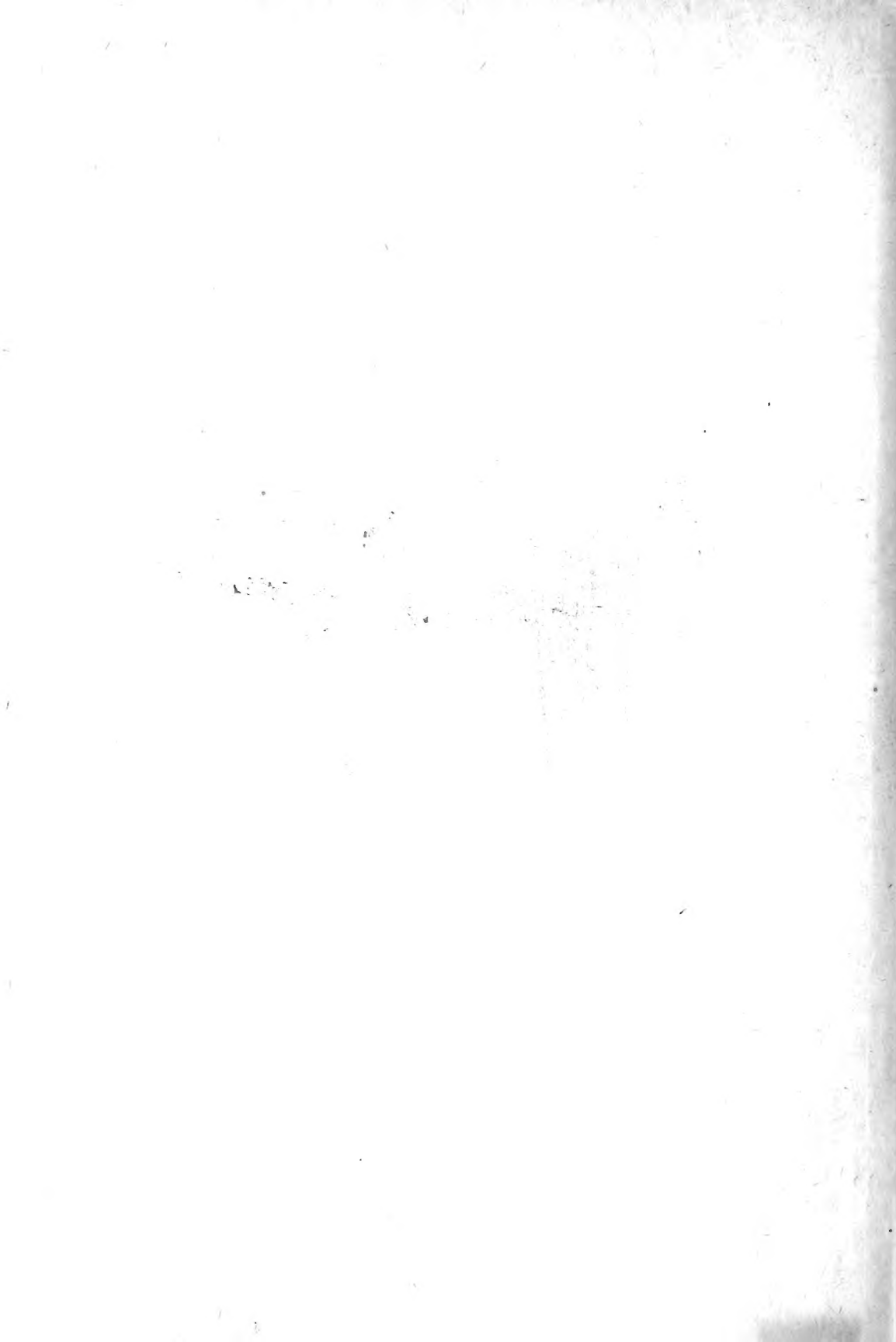
As Conklin ('08) has so well set forth in a recent address, "the mechanism of heredity is merely the mechanism of differentiation." The questions with which we have to deal are those as to the nature of the determining conditions and of the processes, by which the constitution of the cell changes. Perhaps the most direct study of heredity possible in the Metazoa is such a study as Conklin is making of the internal determining conditions in the differentiating cells of the developing organism. When one comes to the study of heredity in the Protozoa, this simply coincides with a study of the determining causes of differentiation.

Johns Hopkins University
Baltimore, Md.
March 10, 1908

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